# IEA / ENFOR

1984:4

Joint Report

Environment Canada | Environnement Canada

# The Use of Root Symbioses in Intensive Forestry

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Canada

FORESTRY ENERGY AGREEMENT
INTERNATIONAL ENERGY AGENCY

ENVIRONMENT CANADA
CANADIAN FORESTRY SERVICE

# THE USE OF ROOT SYMBIOSES IN INTENSIVE FORESTRY

by

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Report No. 4, 1984

IEA/FE, Programme Group 'B'-
Biomass Growth and Production
and
ENFOR
Environment Canada
Canadian Forestry Service

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The IEA/FE PG'B' - Biomass Growth and Production Program is assisted by the Faculty of Forestry, University of Toronto, and by the Canadian Forestry Service and the Ontario Ministry of Natural Resources.

## PREFACE

In April, 1978, a number of countries within the International Energy Agency (IEA) decided to sign an Implementing Agreement (IA) for a program of research, development and demonstration on forestry energy. The work is implemented via Programme Groups and Cooperative Projects.

Work within the group for biomass growth and production has resulted in many reports which are being published in this series. The funds for the publication of these reports have been supplied by the Biomass Energy Project of the National Research Council of Canada, the Ontario Ministry of Natural Resources, and the Canadian Forestry Service.

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Fortin, J.A. and A. Carlisle. 1984. The use of root symbioses in intensive forestry. IEA/FE PG'B' - ENFOR CFS, Report 1984:4. 79 pp. + appendix.

## ABSTRACT

This report describes the biology of nitrogen-fixing and mycorrhizal symbioses, their occurrence on tres and shrubs of potential value in intensive forestry, ways of handling the microbial symbionts, their effect on tree growth and survival, and their interaction with other forest soil organisms. Both nitrogen-fixing and mycorrhizal symbioses, which interact with each other, have considerable potential in intensive forestry and could increase fibre yield and crop security, as well as help to maintain soil fertility.

There are still considerable gaps in our knowledge of both symbioses. However, even where techniques are available, many foresters are not sufficiently informed about soil biology to take advantage of the use of symbioses as management tools, indicating a shortcoming in soil science education at forestry schools. Recommendations are made to test the techniques under forest conditions, investigate the biology of the symbioses, and increase the soil biology content of forestry curricula.

## RÉSUMÉ

Les auteurs décrivent la biologie des symbioses aboutissant à la fixation de l'azote et à la mycorhization, leur fréquence chez les arbres et les arbustes prometteurs pour la foresterie intensive, les moyens de maîtriser les symbiotes microbiens, leur effet sur la croissance et la survie des arbres et leur interaction avec les autres organismes des sols forestiers. Les deux types de symbioses, qui interagissent l'une avec l'autre, recèlent des possibilités considérables en foresterie intensive et pourraient accroître la production de cellulose et la survie des peuplements tout en aidant à maintenir la fertilité du sol.

Nos connaissances des deux symbioses en sont encore à leur début. Toutefois, malgré les techniques voulues, beaucoup de forestiers sont insuffisamment informés de la biologie des sols pour se servir des symbioses en aménagement, ce qui trahit une carence de la formation en pédologie dans les écoles de foresterie. Des recommandations sont faites pour éprouver les techniques en conditions réelles, étudier la biologie des symbioses et accroître le nombre de cours en biologie des sols dans les études de foresterie.

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## INTRODUCTION

The purpose of this report is to inform the International Energy Agency's (I.E.A.'s) member countries about the occurrence, function and biology of root symbioses, including mycorrhizae (ectomycorrhizae and endomycorrhizae) and nitrogen-fixing actinorhizae and Rhizobium associations, their use in intensive forestry systems, and their implications for forest management.

The report will summarize present knowledge of nitrogen fixation and mycorrhizae, and deal in detail with symbioses relevant to forest management. Root symbioses occur in most families of plants and these will be discussed in general. However, emphasis will be placed on tree genera previously reported in an earlier IEA report (Lavoie and Vallee, 1981) as being of potential value for forest biomass production. These include Abies, Acacia, Acer, Ailanthus, Alnus, Betula, Caragana, Castanea, Catalpa, Fraxinus, Gleditsia, Larix, Liquidambar, Liriodendron, Nothofagus, Paulownia, Pinus, Platanus, Populus, Prunus, Quercus, Rhus, Robinia, Salix, Sequoia and Ulmus.

Geographically, the report will focus on forestry in the temperate forests of Europe and North America, but New Zealand will be included and mention will also be made of relevant tropical situations.

## BACKGROUND

### Pressures on the Forest Resource

In recent years, the pressures on the world's forest resources have increased considerably. Not only are the forests providing more wood for conventional forest products such as lumber, pulp, particle board, etc., but a world-wide energy crisis has directed attention on forests as a source of fuel. This is not a new idea and developing nations still rely on wood as a solid fuel for most of their domestic cooking and heating. What is new is that developed nations, the major energy users, are examining wood fibre as a source of liquid and gaseous fuels to at least partially replace fossil fuels which have been their major energy source for half a century. In addition, wood-burning stoves are again being used in some developed nations to supplement costly electric, oil and gas heating. Microbiologically transformed wood is also being considered as a potential source of food for cattle and other animals.

It can be assumed that since the world's population is still increasing, these pressures will continue to increase.

### Increasing Yield

The challenge of providing more wood fibre from the forests is being met by forest managers, loggers, and forest scientists who are seeking more efficient, intensive silvicultural and logging systems that will produce more fibre per unit of land area on sites with reasonable access.

A number of management and logging systems have been proposed to increase yield:

Logging Techniques

(a) harvesting stems, branches and leaves of commercial species

(b) harvesting stems, roots, branches, and leaves of commercial species

(c) total site harvesting (all stems, branches and leaves of commercial and non-commercial trees and all shrubs)

(d) mechanization of harvesting and on-site chipping

Management Techniques

(a) use of rapidly-growing species

(b) use of improved genotypes

(c) use of shorter rotations (5 to 30 years)

(d) use of better site preparation

(e) utilization of fertile soils, not normally used for forestry, in agroforestry systems

(f) increased use of mechanization

(g) increased use of chemicals to control weed competition (herbicides) and improve nutrient supply (fertilizers)

In some areas it is intended that intensive logging (eg. whole-tree harvesting) will be used in conjunction with intensively-managed stands grown on short rotations.

These logging and management techniques can, and will, increase the yield of wood fibre, but costs will increase correspondingly. The economics of some of these operations have been questioned, but few managers have questioned the biological feasibility and desirability of disturbing sites at frequent intervals, removing more wood fibre per rotation, and taking out branches and twigs.

## Environmental Considerations

Increasing the frequency of disturbance of forest soils, harvesting more fibre per hectare, and reducing the site recovery time between harvests will inevitably increase stresses on the soil. Foresters are in a similar position to that of agriculturists who, in recent years, have been asked to increase yields on soils of limited productive potential. Forest soils are generally of low fertility and can only produce so much without considerable, costly interference by man to maintain or upgrade the productive potential of the site. The more intensive the level of cropping and management becomes, the more energy man needs to supply to maintain the soil and sustain the yield of both high and low fertility soils.

A great deal of attention has been focussed on the potential drain of nutrients from forest soils caused by increasing yields, harvesting stems and branches, and shortening rotations. Scientists have concentrated their attention on nutrient inventories and budgets, estimating the quantities of nutrients in the soil, the trees and the total forest, and computing how much will be taken out by different harvesting intensities (stems, stems and branches, etc.) at different rotations. They have estimated the income of nutrients from precipitation, mineral weathering, etc. to compute total nutrient income. Finally, they have calculated whether nutrient income per rotation is more or less than the outgoing nutrients.

In general, as one would expect, the results show that on long rotations (over sixty years), harvesting stems and branches does not often cause a major drain of the bases (calcium (Ca), potassium (K), magnesium (Mg)) in temperate forests but may reduce some elements, such as nitrogen (N). However, shortening rotations can cause considerable nutrient losses. The impact of whole-tree harvesting in tropical rain forest, where most of the soil nutrient capital is in the litter and vegetation, is considerable.

Although these budgeting studies have provided a valuable information base, they have not provided much guidance for the forester. The variability of the results and their often inconclusive nature have lulled forest managers into a sense of false security. The effects of intensive logging and management do not seem to be too bad, at least for some elements, for long rotations. Substantial nitrogen losses seem likely on shorter rotations, but the forester often feels that fertilizers can readily correct any imbalance.

Unfortunately, budgeting studies do not tell the forester how much of a loss is significant or serious. Also nutrient budgets do not give a complete picture. Increased carbon losses from the site due to removal of wood fibre are likely to reduce soil organic matter levels and ion exchange capacity. In addition, frequent site disturbance and clearing is likely to increase organic matter mineralization, nitrification and possibly denitrification. The forest system is very complex, and the effects of forest management and harvesting can only be assessed, even approximately, by interactive, predictive, simulation models (eg. FORCYTE 10 and 11) which are still scarce and expensive to operate.

Taking into account all the shortcomings of nutrient inventory and budget studies, and nutrient losses due to site disturbance, it is very likely, in fact almost certain, that nitrogen will be one of the major nutrients lost to the forest ecosystem. it can be replaced by nitrogen in rain, snow, aerosols and particles in the atmosphere; by free-living, nitrogen-fixing organisms in the soil; by symbiotic associations of bacteria with higher plants; and by fertilizers. The forester has these tools to manage the nitrogen in forest soils. Traditionally he has used nitrogen fertilizers, but these are costly in terms of both money and the energy required to manufacture them.

## Efficient Use of Soil Nutrients by Trees

Trees vary considerably, even within a species, in the efficiency with which they use soil nutrients. Tree breeders are aware of this and attempts have been made to select and breed genotypes with a superior ability to use soil nutrients efficiently. The efficiency of uptake is determined by many tree characteristics, including root surface ion exchange capacity and root surface area. Breeding for enhanced nutrient use takes many years and is very difficult since such a complex characteristic is likely to be controlled by many genes.

Nutrient elements in the soil can be found in both available and unavailable forms. They also vary in their mobility. Potassium ions, for example, are mobile and can diffuse rapidly in the soil solution. In contrast, phosphate ions diffuse very slowly, and a zone of phosphorus (P) depletion can occur in the immediate vicinity of tree roots that are actively taking up elements. During diffusion, any phosphate ion that comes in contact with clay particles is likely to be adsorbed and immobilized. In the presence of cations such as calcium and aluminium (Al), the phsphate may revert to relatively immobile rock phosphate.

During evolution, more extensive root systems were developed to deal with this problem of depletion of available phosphorus, but this requires diversion of energy resources. Symbiotic fungi (mycorrhizae) also evolved and helped to improve phosphorus uptake by increasing the effective absorbing root surface and penetration of soil microsites. Trees genetically capable of forming effective mycorrhizae take up phosphorus more efficiently than those that do not have this attribute. The availability of elements in the soil is governed by the compounds there and their complex interaction with the soil microorganisms, the chemical and physical characteristics of the soil solution, colloids and gases, and the soil environment. All, except possibly the latter, can be chemically or biologically manipulated by the forester.

## Restoration of Soil Fertility

While foresters have been involved for centuries in forest management to regenerate forests and sustain yield, forest soil management has not been incorporated in many management plans. In some cases, the low intensity of management and harvesting (stem only) has meant that forest soil degeneration has not been a major problem except for erosion on some logged sites. However, with intensified management the risk of soil degradation could be a constraint.

The development of adequate soil management techniques has been impeded by the limited information available about forest soil physics, chemistry and biology, and the dynamic interaction of the soil, vegetation and fauna. Compared with agriculture, forest soil science is still in its infancy.

The forester has a number of tools with which he can manage his soils and improve the efficiency with which trees use the nutrient elements present. These are:

i. the vegetation

ii. site preparation

iii. fertilizers

iv. microorganisms

The vegetation cover can be rotated using coniferous and deciduous tree species with very different leaf litter characteristics that affect the soil pH, the rates of decomposition, and the mobility of nutrients in the soil profile. The site can be prepared by scarification to obtain a short-term increase in available nitrogen, and the vegetation can be burned to provide a surface dressing of bases (calcium, potassium, etc.). The forester can apply many types of slow- and rapid-release fertilizers to supply nitrogen, phosphorus, calcium, potassium, magnesium, sulphur (S), and trace elements. Also, microorganisms in the soil can be used to regulate the level and characteristics of available nitrogen, manipulate the phosphorus cycle and increase the uptake of phosphorus by the trees when this element is tightly bound in a slow-release form.

The following will focus on fertilizers in brief, and the use of microorganisms in more detail.

Forest Fertilization

Foresters are aware that in many parts of the world, adding nitrogen to a forest soil results in considerable increases in yield (Ballard, 1979). This is particularly the case in North America where large quantities of urea formaldehyde have been applied with positive effects on tree growth. However, this is not always the case. On some soils, some peats for example, application of nitrogen can reduce growth.

Many empirical fertilizer trials have been carried out throughout the world and nitrogen in either inorganic (ammonium nitrate) or organic form (urea etc.) often gives the best growth responses. Phosphorus also frequently improves yield, but since it is often applied as superphosphates which contain large amounts of calcium, it is sometimes difficult to differentiate the effects of the two elements on tree growth.

Unfortunately, fertilization with nitrogen is very costly and frequently wasteful. When nitrogen fertilizers are added, losses by volatilization and leaching are often heavy. Konkles (1973) reports losses of 50% to 60% of the fertilizer nitrogen either in gases or in solution, and De Bell (1979) reports losses of over 30% of nitrogen from urea applications just from the exit of gases. Consequently, a great deal of the investment in nitrogen fertilization may be lost.

Most fertilizer experiments are empirical and while some are designed to study interactions between elements, very few take into account effects on and interactions with the fungal, bacterial and faunal components of the soil biota. Hill et al. (1975) found that applying fertilizers can affect soil pH, with considerable effect on the soil microflora and microfauna involved in the vital soil processes governing nutrient availability.

The increased growth of trees associated with application of nitrogen fertilizers is often interpreted that all is well in the soil following application. This may not necessarily be the case. These fertilizers can, in some circumstances, prime the soil nitrogen "pump", because soil microorganisms can be stimulated by nitrogen as well as the trees. This can result in excessive mineralization of soil organic matter, nitrification and possibly denitrification, with considerable nitrogen and organic matter loss from the system. Every gardener is familiar with the rapid degradation of soil texture due to losses of organic matter following repeated application of ammonium nitrate or urea.

The organic matter, nutrient elements and micro- and macro-organisms in the soil are in a state of balance. Injudicious application of any nutrient element can have undesirable effects on the soil. Fertilizers are forestry tools that should be used with great caution, for economic and biological reasons, by a responsible manager concerned with the long-term productivity of the soil.

Fertilizers are not the whole answer to soil degradation. If the soil organic matter has been greatly reduced, the soil ion exchange capacity and ability to withstand stress will decline, and elements added will not be retained in the system. Organic matter is difficult to replace. Quite apart from the environmental problem of water pollution by nitrates etc., heavy, unthinking use of fertilizers is not an economical way to maintain forest soil fertility. Fertilizers will always be needed but should be regarded as a supplement to biological techniques.

Biological Techniques

Within the soil is a vast population of organisms, many of which are involved in the soil processes vital to soil fertility and plant growth. The macrobiota include earthworms, molluscs, and arthropods, etc.; the mesobiota include nematodes, springtails, and small arthropods; the microbiota consist of microorganisms such as algae, protozoa, bacteria and fungi. These groups form a dynamic, integrated system (Richards, 1974).

All of these soil organisms interact with each other. Some are free-living and others are bound in symbioses with higher plants. Free-living organisms include bacteria that are vital to the processes of mineralization of the soil organic matter, nitrification and denitrification. Their activity determines the form and availability of nutrients such as nitrogen.

At the interface between the roots of higher plants is a zone of highly-active, free-living bacteria, fungi, protozoa and algae, known as the rhizosphere, where the organisms feed on surface tissues of the roots, on root exudates, and on each other. In this zone, the relationship between the higher plants and the organisms is not usually regarded as symbiotic. The occurrence of bacteria in this zone is ten to fifty times that in non-rhizosphere soil (Dobereiner, 1974); some rhizosphere bacteria fix nitrogen (Richards, 1973).

Symbiotic bacteria are involved in the process of fixation of inert atmospheric nitrogen in an ammonium form that plants can use, and symbiotic fungi are involved in the formation of mycorrhizae. In this context the term "symbiosis" is used in the sense of intimate mutual association. This report will be mainly concerned with nitrogen-fixing microorganisms and mycorrhizal fungi.

## THE NEED FOR BIOLOGICAL TECHNIQUES IN FOREST SOIL MANAGEMENT

The rising cost in dollars and energy required for chemical fertilizers, herbicides, fungicides, and insecticides, and the many environmental hazards associated with them, have stimulated scientists to seek better ways of managing farm and forest soils and controlling competition from pests and diseases.

Already, biological control techniques using viruses and bacteria have been developed to control insect pests. In time, studies of allelopathy will undoubtedly reduce the need for herbicides to control competition. Throughout the world, scientists are finding ways to manufacture food, extract mineral ores, improve the soil and synthesize drugs by manipulating microorganisms.

The manipulation of microorganisms to manage forest soils has high potential. Once the techniques have been developed, it could reduce energy and dollar costs, maintain levels of nitrogen and organic matter, improve ion exchange capacity and phosphorus uptake, make the trees better able to withstand water and nutrient stresses, and increase yield and site productivity by using existing or modified tools provided by nature for these purposes.

## NITROGEN FIXATION

### The Global Context

Nitrogen fixation is a process whereby nitrogen gas in the dinitrogen ($N_2$) form in the atmosphere is converted into a combined ammonia form ($NH_3$3). The process is carried out by microorganisms with specialized enzyme systems capable of reducing the nitrogen. These organisms exist in the free-living state and in intimate symbioses with higher plants. Atmospheric nitrogen is also converted to combined nitrogen oxide forms by natural electrical discharges (lightning), ultra-violet radiation in the upper atmosphere, as well as by man's technology (combustion in internal combustion engines, electrical discharges in generators, etc.). These non-microbial processes account for about 10% of the total combined nitrogen input from atmospheric nitrogen. The nitrogen fertilizer industry produces about $3 \times 10^7$ tonnes of combined nitrogen annually, about 15% of the total combined nitrogen input. Most of the remainder, about 70% of the input, is due to nitrogen-fixing microorganisms, either free-living or in symbioses with higher plants (Postgate, 1978).

The total nitrogen in all the earth's biota (plants and animals) is very approximately $1.5 \times 10^{10}$ tonnes, and the earth's nitrogen cycle transfers very approximately $10^9$ tonnes of this nitrogen annually. The annual input of combined nitrogen to the soil and bodies of water is about $2 \times 10^8$ tonnes of nitrogen, and nitrogen fixation accounts for most of this total. It has been calculated that denitrification processes, whereby nitrate (produced via ammonification of soil organic matter and subsequent nitrification) is converted into atmospheric nitrogen ($N_2$) and nitrous oxide ($N_2O$) gases, are responsible for an approximate loss of $2 \times 10^8$ tonnes $year^{-1}$ of biological nitrogen as gases from the soil and bodies of water. Nitrogen fixation plays a major role in returning this nitrogen to the earth's biomes. This process is, by it's role in the global nitrogen cycle, essential to life on earth as we know it.

## Nitrogen in Plants

Nitrogen is an important plant and animal component. It is present in amino acids which, linked by peptide bonds, are the building blocks of proteins. The element occurs in cell RNA and DNA, in chlorophyll, cytochromes and vitamins. Animals assimilate nitrogen via plants, and plants take up nitrogen from soil or water as nitrate ($NO_3^-$) and ammonium ($NH_4^+$) ions, some plants preferring one ionic form more than the other (Van Den Dreissche, 1971). Biological nitrogen fixation produces, via ammonia ($NH_3$) and amino acid synthesis, combined nitrogen in a form which plant hosts can use directly when in symbiosis with nitrogen-fixing organisms or indirectly following death and decay of free-living, nitrogen-fixing organisms. Ammonia is the main nitrogen source for plants in terms of nitrogen accumulated per unit weight (Delwiche, 1979).

## Nitrogen in the Soil

Nitrate ($NO_3^-$) ions in the soil tend to be lost from the soil system by leaching. Positively-charged ammonium ($NH_4^+$) ions tend to be immobilized in soils by negatively-charged clay particles. The ammonium form is preferable from a soil nitrogen conservation point of view, and this is the form of combined nitrogen produced, via ammonia, by nitrogen fixation.

The fixation of nitrogen by soil bacteria takes place in the context of the soil's nitrogen cycle. Organic residues containing proteins are decayed aerobically by several microorganisms to form ammonia (ammonification, i.e. organic matter $\rightarrow NH_2 \rightarrow NH_3$) which may be lost as a gas or, in aerobic conditions, converted by Nitrosomonas spp. to nitrite ($NO_2^-$) with a release of energy, and then to nitrate ($NO_3^-$) by Nitrobacter spp. If conditions are anaerobic, denitrification is carried out by many organisms such as Pseudomonas spp. which produce nitrogen ($N_2$) and nitrous oxide ($N_2O$) gases, which can escape from the system and be lost. The nitrogen-fixing organisms fix nitrogen gas in the soil, and contribute ammonium ions directly or indirectly (via decaying proteins, etc.) to the nitrogen cycle.

## Nitrogen-Fixing Organisms

Nitrogen-fixing organisms exist as free-living microorganisms or in symbiotic association with fungi and higher plants.

Free-Living Nitrogen-Fixing Organisms

Free-living, nitrogen-fixing organisms are those which assimilate elemental nitrogen and combine it with hydrogen without forming symbioses with other organisms. They include bacteria, such as Aerobacter, Azotobacter, Beijerinckia, Chromatium and Clostridium; actinomycetes, such as Nocardia; and blue-green algae, such as Anabaena, Calothrix, Fischerella, Klebsiella and Nostoc. Some of the free-living, blue-green algae may form nitrogen-fixing associations with fungi to form lichens (eg. Peltigera), with bryophytes (eg. Blasia), pteridophytes (eg. Azolla), gymnosperms (eg. Encephalortos) and angiosperms (e.g. Gunnera), but are not dependent upon the association. Bacteria in leaf nodules in plants such as Psychotria and Pavetta fix nitrogen in a semi-symbiotic association and can exist in the free state (Mulder and Brotonegoro, 1974; Stewart, 1970, 1975; Dobereiner and Day, 1975).

These free-living organisms vary in their efficiency, which is generally much less than for those in symbiotic association with higher plants (Table 1).

Table 1: Efficiency of Nitrogen Fixation

| $N_2$-Fixing Bacteria | $N_2$ fixed per gram of compound consumed (mg N) |
|---|---|
| Free-living Azotobacter | 10-20 |
| Klebsiella | c.5 |
| Clostridium | 5-10 |
| Symbiotic | |
| Pea-Rhizobium | 270 |
| Clover-Rhizobium | > 270 |

(from: Mulder, 1975)

Organisms in Symbioses with Higher Plants

Nitrogen-fixing bacteria (including actinomycetes) form symbioses with higher plants. The microorganisms provide the combined nitrogen in a form the higher plants can use, and the plant provides carbohydrate energy from photosynthesis and protection for the organism. The largest group of such symbioses occurs in the legumes (eg. beans, peas, clovers, alfalfa, lupins, etc.) which are important economic crops. They include tree and shrub species such as Robinia, Caragana, Cercis, Cytisus, and Gleditsia that grow in the temperate zone, together with many tropical tree and shrub species (eg. Leucaena, Acacia, Albizia, Sesbania, Acrocarpus, Calliandra, Dalbergia, Mimosa, Enterolobium and Samanea). Most of these tropical trees are very fast-growing (National Academy of Sciences, 1979). The bacterial symbiont is Rhizobium which is located in root nodules.

Non-leguminous trees and shrubs such as Alnus, Casuarina, Ceanothus, Elaeagnus, Myrica, Hippophae, Purshia and Comptonia also exhibit nitrogen-fixing symbioses. The symbiont is usually the actinomycete Frankia which occurs in root nodules, but other actinomycetes might be involved.

## The Biology of Nitrogen Fixation

Nitrogen fixation is the anaerobic reduction of molecular nitrogen gas ($N_2$) by hydrogen to ammonia by the enzyme nitrogenase.

## The Biochemical Process

The two nitrogen atoms that form the dinitrogen molecule ($N_2$) are linked by a stable triple bond ($N \equiv N$) which makes the $N_2$ molecule unreactive and inert. In the industrial Haber-Bosch process, red-hot magnesium or a catalyst at high pressures is necessary to make the $N_2$ reactive. Thus, a great deal of energy is required to disrupt the triple bond.

Nitrogen-fixing microroganisms are able to make the dinitrogen molecule reactive in water at ordinary temperatures and pressures, because of the exceptional properties of the enzyme nitrogenase (Postgate, 1978). The energy for the reaction is provided by ATP (adenosine triphosphate) which loses energy when hydrolysed to ADP (adenosine diphosphate).

In brief, the reaction is:

$$N_2 + 3H_2 \xrightarrow[\text{energy ATP}]{\text{nitrogenase}} 2NH_3$$

This takes place in high oxygen tensions (i.e. low oxygen partial pressures).

The enzyme nitrogenase has a number of specific properties (Postgate, 1978):

1. it consists of two proteins, azoferredoxin (an Fe protein) and molybdoferredoxin (an MoFe protein), and contains iron (Fe) and molybdenum (Mo)
2. it is destroyed by oxygen
3. it needs magnesium ions to be active
4. it converts ATP to ADP with the emission of energy that can be used in the nitrogen-fixing process
5. it is inhibited to ADP
6. it reduces $N_2$ (i.e. $N \equiv N$) and several other triple-bonded molecules
7. it reduced hydrogen ions ($H^+$) to gaseous hydrogen molecules ($H_2$)

The need for low oxygen levels is a major constraint, and the inhibition of nitrogenase activity by ADP (the end product of ATP - ADP) is an inbuilt regulator of the enzyme. The reduction of hydrogen ions ($H^+$) to dihydrogen gas ($H_2$) means that hydrogen can be lost from the system if the hydrogen ions are not linked to the nitrogen, a potential waste of ATP energy.

The ATP energy source consists of ATP molecules containing magnesium (MgATP). The electron transfer process and the MgATP appear to be linked to the Fe-protein site where the MgATP is hydrolysed to ADP. This releases energy which permits the $N_2$ reduction process to begin. Molybdenum is the specific catalyst for the reduction, and the MoFe-protein is necessary for the continuation of the process; this MoFe-protein appears to be the important reduction site (Burns and Hardy, 1975; Ljones, 1974; Schrauzer, 1977; Stiefel, 1977; Watt, 1977). Optimal conditions for nitrogen fixation are at oxygen tensions of not more than 0.35 atmospheres, an average temperature of about 25°C and nearly neutral pH (Burns and Hardy, 1975). Low temperatures can effectively inhibit nitrogenase activity.

Energy Sources

The need for energy to fix nitrogen is another major constraint on fixation of nitrogen by nitrogenase. Free-living, nitrogen-fixing organisms without chlorophyll rely on the soil for energy, and the nitrogen they fix reaches plants only slowly as the organsims die and decay. Blue-green algae contain chlorophyll and can supply the nitrogen-fixing process with energy-rich photosynthates. These organisms slowly release the fixed nitrogen for use by higher plants. Microorganisms such as Rhizobium and Frankia, which form symbioses with higher plants, are supplied with energy from the host's photosynthesis and also release the fixed nitrogen to the host plant in a form the latter can use in its metabolism with minimum delay. This reflects the efficient intimacy of microorganisms and host in symbiosis.

In the symbioses, the host plants pay an energy price for their supply of microbially-fixed nitrogen. The plant photosynthates used to provide energy for the reduction of the nitrogen could be used for other purposes, such as growth. It is a matter of energy costs and benefits.

Hydrogen Losses

Microorganisms vary considerably in their ability to fix nitrogen, and many are very specific for a particular host plant. This provides geneticists with a promising field for strain selection. Any nitrogen-fixing reaction that "leaks" considerable amounts of hydrogen is inefficient and wastes energy as far as the plant in concerned. Cell-free nitrogenase will release hydrogen from water if nitrogen or a similar substance is not available, a side-reaction which is a drawback. Some nitrogen-fixing bacteria can prevent this hydrogen loss but some cannot. Klebsiella and Clostridium pasteurianum evolve hydrogen during anaerobic growth, which may account for their low ATP efficiency. On the other hand, Azotobacter does not usually evolve hydrogen. It contains the enzyme hydrogenase which can be reversible (catalyzing hydrogen evolution or uptake). However, the form of hydrogenase in Azotobacter only

catalyses hydrogen uptake, effectively reducing hydrogen loss. If hydrogenase activity is inhibited by acetylene or carbon monoxide, Azotobacter evolves hydrogen from the nitrogenase system (Postgate, 1978). Hydrogen loss can be significant in crops of legumes. For example, in garden peas and soya beans, 39% to 55% respectively of the nitrogenase activity is diverted to wasteful hydrogen evolution (Reporter, 1978). This has attracted the attention of scientists seeking a way to produce hydrogen fuel without large energy inputs from coal, oil, gas or electricity, but little progress has been made.

Control of Oxygen

The fact that nitrogen fixation can take place on a planet with an atmosphere rich in oxygen means that the organisms, and in some cases the hosts, have had to develop a number of strategies to remove oxygen from the site of nitrogenase activity. In the case of aerobic nitrogen-fixing microorganisms, oxygen must be allowed to enter the bacteria to permit multiplication and ATP formation without the oxygen impeding the nitrogenase system. Nitrogen-fixing blue-green algae have the additional problem that their photosynthesis produces oxygen which must be excluded from the nitrogenase.

Obligate anaerobes such as nitrogen-fixing Clostridium pasteurianum or Desulfovibrio desulfuricans, which reduce sulphates, avoid the oxygen problem. D. desulfuricans ceases nitrogen fixation in air, but C. pasteurianum only has its nitrogenase system damaged if the cells are broken up. Facultative anaerobic bacteria can grow aerobically or anaerobically, and if they can fix nitrogen they do so in anaerobic situations (e.g. Klebsiella, Enterobacter, Bacillus). Other nitrogen-fixing bacteria (e.g. Beijerinckia) form cell coats of polysaccharide slime to impede oxygen ingress. In some cases, the nitrogenase enzyme seems to protect itself by assuming a molecular conformation that is inactive, is not damaged by oxygen, and can revert to the active form when oxygen tensions increase. Another strategy to exclude oxygen from the nitrogenase site is the formation of heterocysts in blue-green algae. Anabaena, for example, has these sub-cellular compartments at regular intervals along the algal filaments when the organism is fixing nitrogen. These compartments isolate the nitrogenase from the photosynthesizing (oxygen emitting) parts of the filament, preventing damage to the nitrogenase.

The most complex oxygen exclusion system occurs in the nodules on the roots of nitrogen-fixing legumes associated with Rhizobium. Many nodules are pink due to the presence of a "heme" (or haem) compound, a haemoprotein similar to mammalian haemoglobin. Like haemoglobin, this heme compound, leghaemoglobin, has a very high affinity for oxygen, and manages to provide enough oxygen for the Rhizobium's metabolism without harming the nitrogen-fixing nitrogenase. The host plant is mainly responsible for producing the leghaemoglobin. Although Rhizobium's nitrogen-fixation activity is most efficient when associated with a host plant, it can fix nitrogen in isolation in vitro if oxygen tensions are artificially reduced. This ability to fix nitrogen outside a host plant appears to be restricted to slow-growing rhizobia; fast-growing rhizobia have not yet been induced to fix nitrogen outside the host plant.

The nodules on the roots of nitrogen-fixing non-legumes symbiotically associated with the actinomycete Frankia do not work in the same way as the legumes. Frankia appears to be more or less autonomous for its control of high oxygen tension at the site of nitrogenase activity, and a heme compound is apparently not involved. The precise mechanisms are not yet understood.

This apparent autonomy of Frankia is important to geneticists. Attempts to transfer the nitrogen-fixing Rhizobium system to non-fixing plants is impeded by the fact that the new host must be manipulated to accept the Rhizobium and also develop the mutual leghaemoglobin oxygen transfer system, involving many genes. The autonomous Frankia does not need the leghaemoglobin, and transfer to other tree species should be simpler. The reader is referred to Quispel (1974) for more details.

## Legumes

The family Leguminosae, the legumes, is one of the largest families of flowering plants in the world. It consists of about 650 genera and 18,000 species widely distributed in temperate zones, the humid tropics, dry sites, highlands, lowlands, and in water. The family has three subfamilies:

1. Caesalpinoideae - about 2800 trees of the tropics and savannahs

2. Mimosoideae - about 2800 species that are mainly small trees and shrubs of tropical and subtropical regions, including North America

3. Papilionoideae - about 12,000 species, mainly herbs, with worldwide distribution (National Academy of Sciences, 1979)

Although legumes are most abundant in the tropics where they have considerable untapped potential for wood fibre, food and grazing, the family also includes herbs, shrubs and a few trees that could be used in forestry in temperate zones. Legumes such as white clover (Trifolium repens), lucerne (Medicago sativa), soybeans (Glycine spp.), broad beans (Vicia faba) and french beans (Phaseolus vulgaris) are widely used in agriculture and horticulture in Europe and North America. Legumes occur naturally in the forest flora (e.g. vetches); they are common as pioneer plants on infertile sites such as sandy soils, road verges, gravel, and have been used to stabilize steep, infertile banks. Their ability to fix nitrogen has been used to improve soils and colonize sites deficient in nitrogen.

Many soils contain free-living Rhizobium species which do not usually fix nitrogen. They are attracted to legume roots and infect the hosts, accumulate in the cells, produce nitrogenase and fix nitrogen. If, however, the soil is rich in combined nitrogen, the host plants do not permit infection by the microbe, and symbiotic nitrogen fixation does not take place. Even organic residues left from a previous crop can inhibit infection and nitrogen fixation.

Use of legumes, therefore, requires careful planning and management by both farmer and forester. The latter is less likely to encounter the problem of inhibition of infection by high nitrogen levels, since forest

soils are seldom very fertile. If, however, very fertile soils are used in agroforestry, mini-rotation systems, and legumes are used as soil improvers, this inhibition should be remembered.

Legumes of Potential Value in Forestry

Legumes have potential value in forestry as soil improvers and sources of fibre. The main potential for legume fibre is in the tropics where there are many tree species. In the temperate zone there are far fewer woody species.

Legumes, such as clovers, have been used for many years in forest nurseries, but they may have wider application in forestry. Agricultural legumes, with their Rhizobium symbiont, fix nitrogen well on fertilized soils of high lime content, provided nitrogen levels are not too high. Their performance and management in forest soils has not been explored in any detail, but it is likely that many agricultural herbs will be of little value. Phosphorus is essential to the nitrogen fixation process and forest soils often have very small quantities of this element in a form that is readily available to plants. Haines and De Bell (1979) have compiled a list of legumes of importance in agriculture that will grow on forest soils, tolerate low fertility, low pH and drought, or that have been successful in erosion control (Table 2). These include annuals and perennials, and seem most relevant to green manuring, game browse, and erosion control. They have some potential value for soil amelioration in intensive forestry systems which produce biomass on short rotations. The same applies to the legumes native or naturalized in the United States which are listed by Haines and De Bell (1979) as having potential value in forestry (Table 3). While these legumes are capable of growing on forest soils, few have been tested in forestry systems.

Rhizobium Symbioses with Forest Trees that have Biomass Production Potential in the Temperate Zone

Although Rhizobium symbioses are very common on many fast-growing trees and shrubs in the tropics (National Academy of Sciences, 1979), they do not occur on many of the tree species listed by Lavoie and Vallee as having potential for biomass production in IEA countries (Appendix I) (Lavoie and Vallee, 1981). In this list, the Rhizobium nitrogen-fixing symbiosis is confined to two Acacia species, three Gleditsia species, two Robinia species and Caragana. There are also other promising legume species.

Robinia pseudoacacia (black locust) is a large tree, up to 25 m high, and R. viscosa (clammy locust) grows to about 13 m in height in the mid-eastern United States. The Gleditsias (locusts) are also large trees and G. triacanthos grows to heights of 25 m to 45 m. Other Gleditsias not on Lavoie and Vallee's list (e.g. G. texana and G. aquatica) grow to heights of 40 m and 20 m, respectively (Sargent, 1949). These legumes include very large trees, but there does not appear to be much information about their performance in managed forests. Some Acacias and Caragana are shrubby plants.

Table 2: Agricultural Legumes that Tolerate Forest Soils

| Species | Site tolerated or required | Annual (A) or Perennial (P) |
|---|---|---|
| Aeschynomene americana L. | Wet sites; tolerates flooding | A |
| Centrosema spp. | Dry, infertile soils | P |
| Desmodium canum | Moist, well-drained acid soils | P |
| Lespedeza spp. | Eroded, acid soils low in phosphorus | A - P |
| Lotus corniculatus L. | Deep, well-drained loams or clay loams | P |
| Lupinus angustifolius | Slightly acid, sandy loams | A |
| L. hispanicus spp. bicolor | Poorly to moderately well-drained infertile soils | A |
| L. luteus Kell | Infertile, moderately acid-sandy soils | A |
| Medicago sativa L. | Deep, well-drained loams or clay loams | P |
| Ornithopus spp. | Infertile, moist, slightly acid sandy soils | A |
| Trifolium incarnatum L. | Moderately acid, well-drained sands and clays | A |
| T. repens L. | Moist, moderately fertile clay or silt soils | P |
| T. subterraneum L. | Moderately acid, well-drained sandy to loamy soil | A |
| T. vesiculosum Savi | Well-drained, sandy and clayey soils of moderate acidity and low fertility | A |
| Vicia villosa | Sandy to loamy soils | A |

(from: Haines and De Bell, 1979)

Table 3: Native or Naturalized Legumes in the United States of Potential Value in Forestry

| Species | Annual (A) or Perennial (P) |
|---|---|
| Amphicarpa bracteata Ell. | A |
| Apios americana Medic | P |
| Baptisia spp. | P |
| Cassia spp. | A |
| Centrosema virginianum (L.) Benth | P |
| Clitoria mariana L. | P |
| Coronilla spp. | P |
| Crotalaria spp. | A - P |
| Desmodium spp. | A - P |
| Galactia spp. | P |
| Indigofera carolinania (Mill.) | P |
| Lespedeza spp. | A - P |
| Lupinus spp. | P |
| Medicago spp. | A - P |
| Melilotus spp. | A - P |
| Phaseolus spp. | P |
| Rhynchosia spp. | P |
| Schrankia microphylla Macbr. | P |
| Sesbania exaltata (Raf.) Rydb. | A |
| Strophostyles spp. | A - P |
| Stylostanthes biflora L.B.S.P. | P |
| Tephrosia spp. | P |
| Trifolium spp. | A - P |
| Vicia spp. | A - P |

(from: Haines and De Bell, 1979)

These legumes appear to have some potential either as biomass producers or soil improvers or, in the case of Robinia and Gleditsia, as both. Acacias grow in the southwestern United States and Gleditsia grows in the south and southwest, with G. triacanthos growing as far north as the Great Lakes. Caragana is hardy in the severe climate of central Canada.

Other leguminous trees and shrubs of unknown silvicultural importance are listed in Sargent (1949).

Quantities of Nitrogen Fixed

Information about rates of fixation has greatly increased since the development of the acetylene reduction method. Nitrogen fixation can be estimated by measuring nitrogen gas uptake manometrically, or measuring the production of ammonia, the product of the process (Stewart, 1966). These methods tend to be slow. Isotopes of nitrogen ($^{15}N$) have been used to detect nitrogen fixation and this method remains the conclusive test. However, $^{15}N$ techniques are costly.

Nitrogen fixation can be measured indirectly much more easily and cheaply. The methods are based on the ability of the enzyme nitrogenase to reduce substrates other than molecular nitrogen (e.g. nitrous oxide ($N_2O$), cyanide ($CN^-$) and acetylene ($C_2H_2$). A technique has been developed using the reduction of acetylene ($C_2H_2$) to ethylene ($C_2H_4$) by nitrogenase as an estimate of nitrogenase activity. The gases $C_2H_2$ and $C_2H_4$ can be measured at very low concentrations by gas chromotography. The method is sensitive, relatively economical and easy to use (Turner and Gibson, 1980). It is, however, an indirect method and its results need to be interpreted with care and with confirmation by $^{15}N$ assays when necessary. The methods of evaluating biological nitrogen fixation are fully reviewed in Bergerson (1980).

The quantities of nitrogen fixed vary considerably between species. In the case of the legumes, the economically important alfalfa fixes up to 300 kg $ha^{-1}$ $year^{-1}$ of nitrogen, and clovers, soybeans and groundnuts also fix considerable amounts. The shrubby acacias fix as much as 270 kg $ha^{-1}$ $year^{-1}$ (Burns and Hardy, 1975; Fortin et al., 1979) (Table 4). Non-legumes can fix similar quantities, particularly alders (50 to 300 kg $ha^{-1}$ $year^{-1}$), Hyppophae spp. (179 to 198 kg $ha^{-1}$ $year^{-1}$) and Casuarina (50 to 200 kg $ha^{-1}$ $year^{-1}$) (Fortin et al., 1979; Haines and De Bell, 1979).

The quantities of nitrogen entering the system in biological fixation from symbioses are generally large compared with other sources of nitrogen on arable land (Table 5). For example, nitrogen comes down in rainfall at a rate of about 4 to 6 kg $ha^{-1}$ $year^{-1}$, although it can be much more in industrial areas where concentrations of air pollutants are high (Carlisle, 1980). The free-living, nitrogen-fixing organisms, with their limited energy supply, generally fix about 1 to 4 kg $ha^{-1}$ $year^{-1}$, although this varies considerably from site to site. The importance of these free-living nitrogen fixers should not be underestimated. The fixation may be low per unit of land area, but they are active over huge areas.

Table 4: Rates of Nitrogen Fixation by Legumes and Non-Legumes in the Field

| Legumes (*Rhizobium* symbiont) | Nitrogen Fixation rate (kg $ha^{-1}$ $year^{-1}$) | Reference |
|---|---|---|
| *Glycine max* (Soybean) | 57 - 94 | Burns & Hardy in Fortin *et al.* (1979) |
| *Apios americana* (Groundnuts) | 47 - 91 | " |
| *Pisum* spp. (Garden pea) | 85 | " |
| *Lens esculenta* (Lentil) | 85 | " |
| *Vicia* spp. (Vetches) | 85 | " |
| *Trifolium* spp. (Clovers) | 105 - 160 | " |
| *Medicago sativa* (Alfalfa) | 128 - 300 | " |
| *Lupinus* spp. | 150 | " |
| *Acacia* spp. | 270 | " |
| **Non-Legumes (*Frankia* symbiont)** | | |
| *Alnus rubra* | 100 - 300 | Fortin *et al.* (1979) |
| *A. crispa* | 62 - 157 | " |
| *A. rugosa* | 79 - 150 | " |
| *A. incana* | 50 | " |
| *A. glutinosa* | 12 - 30 | " |
| *Casuarina* spp. | 50 - 200 | Haines & De Bell (1979) |
| *Ceanothus* spp. | 60 | " |
| *Coriaria* spp. | 90 - 120 | Fortin *et al.* (1979) |
| *Elaeagnus* spp. | 2 - 15 | Haines & De Bell (1979) |
| *Hyppophae* spp. | 80 - 197 | " |
| *Myrica* spp. | 9 - 15 | " |

Table 5: Mean Fertilizer Usage on Arable Land[1],[2]

| | kg N $ha^{-1}$ $year^{-1}$ |
|---|---|
| USA | 43 |
| United Kingdom | 122 |
| Netherlands | 476 |
| USSR | 27 |
| Spain | 34 |
| Egypt | 133 |
| Kenya | 12 |
| Nigeria | 0.2 |
| S. Africa | 18 |
| Brazil | 13 |
| India | 11 |
| Japan | 155 |

1 Bond, In: Newton et al. (1977)

2 FAO data

As far as the forester is concerned, nitrogen-fixing symbioses can produce the equivalent of up to 300 kg $ha^{-1}$ $year^{-1}$ of fertilizer nitrogen, with the added bonus that they supply the soil with organic matter.

Handling the Rhizobium Symbiont

Ways of handling Rhizobium cultures and infecting host plants in agriculture have been known for many years. Vincent's (1970) review of the techniques is a good basic guide. Rhizobia are recovered from fresh or cold-stored nodules with great care taken to remove contaminants. The bacteria are raised in aseptic conditions in liquid media, including fresh yeast extract and mannitol (or an equivalent cheaper C-compound). Cultures can be maintained on agar slopes, in a dried state or freeze-dried.

Legumes are inoculated with Rhizobium by applying the bacteria to the seed prior to sowing. The rhizobia can be raised in agar or a liquid medium and applied as a soil-peat medium in which a liquid Rhizobium culture has been intimately mixed. The seeds are inoculated either by applying a culture of Rhizobium suspended in water together with about 40% neutral gum arabic (or 100% sugar) as an adhesive, or as a peat slurry (about 25 g of peat to 100 ml of fluid) with sugar or gum arabic. Pelleted seed is also used to protect the inoculum from acid soil or fertilizer. A common method is to suspend a peat culture of Rhizobium in a sticking agent such as gum arabic (about 40%) or substituted cellulose (e.g. 5% methyl-ethyl-cellulose, hydroxypropyl-cellulose, or carboxy-methyl-cellulose), mix it with the seed for about five minutes and then apply a calcium carbonate coat (passing a 300 mesh sieve) by mixing the seeds in lime for one to two minutes.

A good seed pellet should have an even coat, appear dry with no loose lime, and be firm enough to stand squeezing between the fingers or dropping. Pelleting increases the size of the seed and this must be allowed for in the sowing. The seed should be sown immediately after preparation. Pelleting provides rapid, good inoculation in conditions likely to delay germination or inhibit the rhizobia. It is not suitable where prolonged storage is unavoidable after pelleting (Vincent, 1970).

It is vital, of course, that the correct strain of Rhizobium is used for a particular plant.

Survival and Competition in the Soil Biome

Rhizobium symbionts associated with legumes compete with other organisms in the soil, particularly those in the rhizosphere. Even if a superior strain of Rhizobium is selected, cultured and used to coat the seeds and infect the plants and an active nitrogenase system is established, the superior strain is likely to be replaced by less efficient soil rhizobia if nitrogen fixation in inhibited by high nitrogen levels etc. and this inhibition persists.

Many agricultural crops are annual, or semi-perennial, so the strain is only required to associate with its host for one year. After the nodules decay, the strain will be released to the soil and exposed to full competition with the soil biota. Although some of the older and less

efficient nodules on these annuals are partly suberized (thick walled) they are not woody as in the case of perennial, non-leguminous trees (e.g. alder) with Frankia actinorhizal symbiosis. Once an alder root is infected by Frankia, the large nodules can remain active for several years with the symbiont relatively free from microbial competition. No information was found on the longevity and activity of nodules on perennial, leguminous trees and shrubs (e.g. Robinia and Acacia). The nitrogen-fixing organisms in actinorhizae seem to have a distinct advantage over those in the more ephemeral annual legume nodules.

Social and Economic Importance

The undoubted benefits of legumes in agriculture were known in Roman times and probably before, although farmers did not understand the reasons. The inclusion of a legume in the farm crop rotation began in the early seventeenth century in Britain. This practice spread to Europe and increased food production by 40% to 50% in the eighteenth century. Use of legumes is not a new technique. Attempting to use it in forestry can be said to be finding new ways to use a very old wheel rather than reinventing it.

There can be little doubt that with the rapidly increasing demand by agriculture for fertilizers, the escalating costs, the fact that the hydrogen for the industrial combination of nitrogen with hydrogen comes from natural gas (a non-renewable fossil fuel), and the high energy costs of fertilizer manufacture, forestry will not be able to compete with food production for nitrogen fertilizers. Even if forestry could compete, in view of the costs, the waste by volatilization and leaching, and the potential impact on soil and water, it is questionable whether extensive use of nitrogen fertilizers is even desirable.

Farmers have tended to move away from legume rotations and rely on chemicals, but even in intensive farming, with a potentially high cash flow per acre. People are returning to clover rotations and soybean production.

In this context, legumes have an important role to play. Their main value in forestry appears to be as a green crop grown either before, or during, the growth of the tree crop to restore nitrogen in the soil, increase fibre yield and improve soil organic matter. Tree lupins (Lupinus arboreus) from New Zealand, with their high oil content, are candidates for oil seed cash crops that can be grown with trees (Gordon and Dawson, 1979). This particularly applies to the short-rotation and agroforestry situation (e.g. hybrid poplar plantations). The different management techniques that can be used will be discussed below. Legumes can also be encouraged in forests, particularly where enough light is available to permit the growth of understorey vegetation. These techniques are at present largely untested in forestry situations outside the nursery, and strictly controlled experiments are needed to provide more information. Biologically, use of legumes as a nitrogen source makes sense. It remains to be seen whether or not it is practicable.

Inevitably, use of these nitrogen-fixing plants will be subjected to cost-benefit analyses. When this occurs the intangible long-term benefits of improvement of organic matter levels, and consequent maintenance of ion exchange capacity, soil stability and texture should be included on the benefit side of the equation.

## The Non-Legumes (Actinorhizae)

Studies of nitrogen fixation and its commercial use have focussed on the Rhizobium symbioses in the Leguminoseae, a family that includes many plant species suitable for soil improvement, human food and cattle/wildlife grazing. The nitrogen-fixing non-legumes, with their actinorrhizal (Frankia) symbioses, were neglected until relatively recently. Although they include tree species such as alders, these trees were not of much interest to foresters in the temperate zone, and not many plants with actinorhizal symbioses are suitable for food or even grazing.

Due to a realization that intensification of forestry will result in soil nitrogen stress on many sites, and that extensive use of heavy dressings of nitrogen fertilizers is not practical in the current and forseeable economic and energy climate and is not particularly efficient nor environmentally desirable, there has been a surge of interest from foresters in nitrogen-fixing non-legumes in recent years. Bond (1977) points out that using nitrogen-fixing non-legumes for soil amelioration is not practical in intensive agriculture situations (although some Asian farmers use shrubby alders) due to the slow rotation, but has most promise in forestry which is relatively slow-moving compared to agriculture.

Advances in knowledge about the nitrogen-fixing non-legumes (from now on referred to as actinorhizae) have been impeded by difficulties in identifying and studying the symbiont. The actinorhizal symbiont Frankia has only recently been identified by electron-microscopy. Although this has led to a surge of new work (Becking, 1977; Bermudez de Castor et al., 1977; Lalonde, 1977; Normand et al., 1982; Perradin et al., 1963) that has provided valuable knowledge on culturing Frankia, inoculating plants, and basic biology, far less is known than about rhizobia.

### Nitrogen-Fixing, Non-Leguminous, Tree and Shrub Genera and Species

The presence of non-fungal root nodules on non-legumes (Alnus, Hippophae, Myrica, Elaeagnus, Ceanothus, and Casuarina) has been known since the nineteenth century. Since then, nodules have been recorded in about 160 species, including Coriaria, Discaria, Purshia, Dryas, Cercocarpus, Shepherdia, Arctostaphylos and Colletia, and doubtless more will be found (Bond, 1971). Of these 160 nodulated non-legumes, 56 species are known to have symbioses with actinomycetes (Frankia) that fix nitrogen, covering 12 of the 16 nodulating genera. So far, nitrogen-fixing symbioses have not been found in Arctostaphylos and the nitrogen-fixing capability of Cercocarpus is doubtful (Bond, 1976, 1977; Schnitzer, 1979). There are recent reports of nodulation in the genus Rubus in the tropics but no details were located. Even within a nodulating species such as Alnus glutinosa, not all trees produce nodules (about 1.4% do not). Myrica gale is even more erratic in this respect, which may be attributable to site (Bond, 1977). Just because a plant nodulates does not mean that it invariably fixes nitrogen.

Sixteen nodulating genera are listed in Table 6. Those of potential interest in intensive forestry systems as both biomass producers and soil improvers include arborescent species of Alnus and Casuarina. The latter is an Australasian genus (Australian pine) not suited to temperate and north temperate forestry. Cercocarpus from Mexico and the United States includes both small trees and shrubs, but is a grazing genus more than a fibre producer.

Table 6: Genera of Non-legumes Known to Form Root Nodules

| Genus[1] | Family[2] | No. of Species in family[1] | Habit[2] | Distribution[2] | Current Use[1/2] | N-fix ability some spp.[1] |
|---|---|---|---|---|---|---|
| Alnus (Tourn.) L. | Betulaceae | 25 | Tr. & Shr | N. Temp.+ Andes | Wood fibre | + |
| Arctostaphylos Adans. | Ericaceae | 32 | Shr. | N.Temp. Arctic | - | - |
| Casuarina L. | Casuarinaceae | 35 | Tr. & Shr. | Austr; Pacific | Wood fibre[2] | + |
| Ceanothus L. | Rhamnaceae | 40 | Shr. | N.Amer; Understorey sp. | Ornam.[2] Shr. | + |
| Cercocarpus H.B. et K. | Rosaceae | 10 | Shr. Small Tr. | Mexico, USA | Browse[1] | ? |
| Colletia Comm. ex Juss. | Rhamnaceae | 15 | ? | S.Amer. | ? | + |
| Comptonia l'Her. | Myricaceae | 1 | Shr. | N.Amer. | - | + |
| Coriaria Niss. ex L. | Coriariaceae | 10 | Shr. | Medit. to Japan, N.Z., Chile, Mex. | Tan, &[2] Dye | + |
| Datisca L. | Datiscaceae | 2 | Shr. | USA | - | +[3] |
| Discaria Hook. | Rhamnaceae | 15 | Shr. | Andes, Brazil N.Z., Austr. | - | + |
| Dryas L. | Rosaceae | 2 | Shr. | Arctic, Alpine | - | + |
| Elaeagnus (Tourn. L.) | Elaeagnaceae | 30 | Shr. | Asia, Europe | Edible Fr. Cult. Shr.[2] | + |
| Hippophae L. | Elaeagnaceae | 2 | Shr. | N.Temp. | - | + |
| Myrica L. | Myricaceae | 45 | Shr. | Temp; N.Temp. Sub Trop. | - | + |
| Purshia DC | Rosaceae | 1 | ? | USA | Browse[1] | + |
| Shepherdia Nutt. | Elaeagnaceae | 3 | Shr. | N.Amer. Understorey spp. in Rockies | Some spp.[2] Edible | + |

1 Bond (1976, 1977); Burns and Hardy (1975); Fortin et. al (1979)
2 Willis (1951)
3 Winship and Chaudhary (1979)

Note: Tr. = Tree; Shr. = Shrub; Fr. = Fruit; Cult. - Cultivated

The rest of the nodulating genera include nitrogen-fixing shrubs that could be considered as soil improvers, with the exception of Arctostaphylos and possibly Cercocarpus whose nitrogen-fixing capabilities are unknown. Within these shrubby, nitrogen-fixing genera, Colletia is a tropical or sub-tropical plant and is not likely to be suitable for use in temperate countries. According to Bond (1977), Purshia (bitterbrush) is an important browse species in North America. The remaining non-arborescent, nitrogen-fixing genera (Ceanothus, Coriaria, Discaria, Dryas, Elaeagnus, Hippophae, Myrica and Shepherdia) all include species which fix nitrogen and will grow in temperate conditions. Species of Dryas and Myrica are widespread pioneers on very poor sites in the north temperate to arctic zone.

A genus omitted by earlier authors from the nodulated, nitrogen-fixing genera is Comptonia. Comptonia peregrina (L.) Coult. (in Myricaceae) is an aggressive pioneer species on poor sites east of the Great Lakes in Ontario, Quebec and eastern maritime Canada south to Tennessee and Georgia (Scoggan, 1979). It is able to nodulate and fix nitrogen, and improve infertile soils.

Not all species within these genera are known to fix nitrogen. Many species remain untested. Some plants produce nodule-like growths due to fungi (e.g. Podocarpus) or microbial and viral infections, which should not be confused with nodules containing actinorhizal or rhizobial symbioses (Wollum and Davey, 1975).

It seems likely that with the assistance of the acetylene reduction technique and the stimulus of the increased interest in non-legume nodulation and nitrogen fixation, more genera and species will be found to have symbiotic association with Frankia and other actinomycetes.

In the list of trees considered by Lavoie and Vallée (1981) to have potential value for biomass production, the only genus with actinorhizal symbioses (Appendix I) is Alnus. They list thirteen Alnus species, not all of which have been studied for their nitrogen-fixing ability. It is likely, however, that these species are able to nodulate and fix nitrogen to some extent. This list, however, only includes trees and shrubs of potential fibre-producing capability, and does not cover the shrubby or herbaceous nitrogen-fixing legumes and non-legumes.

Quantities of Nitrogen Fixed

The quantities of nitrogen fixed by non-legumes forming actinorhizal symbioses are listed in Table 4. The alders (Alnus spp.) fix the most (up to 300 kg of N $ha^{-1}$ $year^{-1}$), but two other genera (Casuarina and Hippophae) can fix about 200 kg of N $ha^{-1}$ $year^{-1}$. Myrica fixed only 9 to 15 kg N $ha^{-1}$ $year^{-1}$ but even this is greater than the average nitrogen income in precipitation. While other non-legumes fix nitrogen, not all have been tested in field conditions and where they occur irregularly mixed with plants that do not fix nitrogen, it is hard to assess their nitrogen contribution.

The amounts of nitrogen which actinorhizal non-legumes fix are more than the average amounts of nitrogen fertilizer applied to arable lands in the United States, United Kingdom, USSR and Spain (Table 5), and considerably more than nitrogen fertilizer applied in forestry.

The actinorhizal symbioses have the biological potential to contribute sufficient nitrogen to maintain productivity of forest soils but are constrained by silvicultural, ecological and economic considerations.

Handling Actinorhizal Symbionts

Isolation. Although in the past Frankia "isolates" have been produced from crushed nodules without surface sterilization, there has been considerable contamination. A better method is to remove the nodule lobes from the plants either in situ in the field or from uprooted samples, clean with water and blot them dry, and immerse them in a 3% W/V aqueous osmium tetroxide ($O_sO_4$) solution for periods ranging from five seconds to six minutes. (It should be noted that osmium tetroxide ($O_sO_4$) is a highly dangerous toxic substance. Great care should be taken to keep it away from the eyes, nose and throat by using well-ventilated fume hoods designed for that purpose.) The lobes should be rinsed three times in sterile distilled water, and transferred for five to fifteen minutes to sterile PVP - PBS (Polyvinyl Pyrolidone and Phosphate Buffer Saline) solutions at concentrations which depend on the host species e.g. 0.1% W/V PVP for Alnus crispa and 3% W/V PVP for A. rugosa. The lobes can then be crushed in new PVP -PBS solutions and transferred to tubes containing the Q mod B medium containing 5 mg/L of L - $\alpha$ - Lecithin. The Q mod medium is described in Table 7, and more details about isolation of Frankia are available in Lalonde (1979), Lalonde and Calvert (1979), Normand et al. (1982), Lalond et al. (1981) and Baker and Torrey (1979).

After a short time, the Frankia appears as a fluffy, whitish outgrowth on the dark surfaces of the treated nodules. These growths are visible to the naked eye, and they can be examined with a 100x objective of a phase or interference contrast microscope (Normand et al., 1982).

This method was developed for Frankia. There might be other actinomycetes capable of forming nitrogen-fixing symbioses.

Subculture and Infectivity. It is necessary to confirm that the cultures are Frankia and will infect the hosts (e.g. Alnus). In sample tubes, the cultures can be broken up with a needle and syringe, and sub-cultured. Infectivity can be tested in plastic growth pouches by germinating surface-sterilized seeds of the host (e.g. Alnus) in nitrogen-free Crone's Solution in an environment of 15,000 lux/15 hours light with 22°C/17°C (day/night) temperatures, but this may vary with the host. The host is inoculated with the actinomycete culture when root hairs appear. Details are given in Lalonde (1979) and Normand et al. (1982).

Effectivity. The effectiveness of the symbiosis in nitrogen fixation can be tested by sowing surface-sterilized seeds of the host (e.g. alder) on Turface, burned montmorillonite clay, in styroblocks fertilized with nitrogen-free Crone's Solution, and growing them under the same conditions as given above for the transparent plastic pouches. Isolated colony clusters of the actinomycete are washed with PBS, broken up with needle and syringe, and applied to the four-week old, nitrogen-starved seedlings. After twelve weeks the growth, weight and nitrogen content of the plants are measured.

Table 7: Q mod medium

| | Per litre |
|---|---|
| $K_2HPO_4$ | 300 mg |
| $NaH_2PO_4$ | 200 mg |
| $MgSO_4.7H_2O$ | 200 mg |
| KCL | 200 mg |
| Yeast Extract (BBL) | 500 mg |
| Bacto-Peptate (DIFCO) | 5 mg |
| Glucose | 10 mg |
| Ferric Citrate [1] | 1 ml |
| Minor salts [2] | 1 ml |
| $H_2O$, Deionized [3] | 1 l |
| Lecithin | 5 mg - 50 mg |
| Agar (if used) | 15 g |

[1] Citric Acid and Ferric Citrate 1% soln.

[2] 1.5 $gl^{-1}$ $H_3BO_3$
0.8 $gl^{-1}$ $MnSO_4.7H_2O$
0.6 $gl^{-1}$ $ZnSO_4.7H_2O$
0.1 $gl^{-1}$ $CuSO_4.7H_2O$
0.2 $gl^{-1}$ $(NH_4)_6\ Mo_7O_{24}.4H_2O$
0.01 $gl^{-1}$ $CoSO_4.7H_2O$

[3] Deionized to: adjust pH 6.8-7.0 with NaOH or HCl then add Ca $CO_3$ (100 mg)

(from: Lalonde (1979))

The nodules can be excised from the roots of those seedlings and tested for nitrogenase activity by acetylene reduction assay.

Storage. Colonies of actinomycetes can be stored by moving them into 2 ml ampoules with about 0.5 ml of the Q mod medium. The ampoules are frozen at -85°C, dessicated in a freeze-dryer for three hours, and vacuum-sealed (Normand et al., 1982).

Large Scale Application. Although Frankia inocula can be produced in 20 x 150 mm test tubes (Kim-raks Kim/caps disposable kits) with 15 ml of Q Mod B medium (+5 mg $^{-1}$ ∝ lecithin) as the medium, industrial fermentors are necessary for large-scale production. Actinomycete colonies can be collected from the vessels by pipette or Vacutainer syringe on a small scale, but it is necessary to use high speed, continuous flow centrifugation (20,000 g; 75 to 100 ml min $^{-1}$) for large scale operations. The Frankia hyphae are effective but it is necessary to break up sporangial membranes to release infective spores, either by a pressurized syringe for small scale work or a blender for large operations.

The actinomycete Frankia is convenient for use with standard greenhouse watering systems if filters are removed. The hyphae and spores can be suspended in the water and slowly applied to young (seven- to twelve-day-old) host plants, the latter being fed afterwards with nitrogen-free Crone's Solution. One tube of Frankia infects about fifty alder seedlings (Fortin and Perinet, 1981).

These techniques were used to successfully inoculate millions of alder seedlings for use as soil improvers on the James Bay Hydroelectric Project.

Symbiont Selection and Availability. Although research on the genetic variation of the ability of Frankia symbionts to fix nitrogen only began recently, a bank of tested Frankia cultures has been established with Canadian ENFOR funds at the Département des Sciences Forestières, Faculté de Foresterie et Geodesie, Université Laval, Ste. Foy, Québec, under Professor A. Fortin and Professor M. Lalonde). About 75% of the world's Frankia cultures are presently at that location.

## Survival and Competition

A criticism levelled at the use of selected nitrogen-fixing symbionts is that it is not likely that they will survive competition from the many microorganisms in the rhizosphere and the surrounding soil. This may be true to some extent of nitrogen-fixing symbioses with Rhizobium in annual, agricultural legumes, or even in perennial legumes with non-perennial nodules. The tendency is for wild Rhizobium strains to take over in the second or third year. Even so, by that time the symbiosis has served its purpose, nitrogen has been fixed, the plants have grown, and the soil can benefit from the nitrogen and organic matter assimiliated by the plants if the latter are ploughed in.

In the case of the non-legumes, however, the Frankia is likely to remain effective for much longer. The nitrogen-fixing non-legumes with actinorrhizal symbioses are mainly woody perennials, and the nodules also tend to be perennial.

In alders, for example, nodules can remain active for many years and reach diameters of 14 cm or more. It is not known whether a nodule's *Frankia* colony changes its strain during the years or contains more than one strain. However, in view of the fact that once a site on the root is infected by *Frankia* it apparently effectively blocks any secondary infection, this seems unlikely.

## The Distribution of Nitrogen Fixation in Forest Ecosystems

The forest ecosystem contains many micro-niches where nitrogen fixation can take place. Quite apart from nitrogen fixation in the soil, microorganisms fix nitrogen in the phyllosphere, in decaying wood, and in the guts of insects and wildlife.

Jones (1970, 1976) found that nitrogen-fixing organisms are present on the leaves of many tree species in Britain, and appreciable quantities of nitrogen were fixed in the Douglas fir phyllosphere.

Lichens which grow on tree bark, rocks and the soil can fix nitrogen. They include genera such as *Peltigera*, *Stereocaulon*, *Collema* and *Nephroma* which have nitrogen-fixing *Nostoc* spp. and *Stigonema* spp. as the algal component of the algae/fungus symbiosis (Henriksson, 1971). Granhall and Linberg (1978) report that microorganisms in the litter layer of *Sphagnum* communities can fix as much as 37 kg $ha^{-1}$ $year^{-1}$, a considerable amount.

Even insects, such as termites, which feed on wood fibre low in nitrogen, supplement their nitrogen intake by the activity of microorganisms in their hind guts (French, 1980; French *et al.*, 1976). Many herbivores are known to have nitrogen-fixing bacteria in their rumen, but evidence indicates that the nitrogen contribution from this source is small.

There is little data for the total income of biologically-fixed nitrogen in forests since not all the niches have been examined. However, together with fixation by free-living organisms in the soil, they are likely to contribute at least small, but appreciable amounts equal to, or more than, the income of nitrogen in rainfall (4 to 6 kg $ha^{-1}$ $year^{-1}$) (Carlisle, 1980).

The quantities of nitrogen fixed in these niches are not comparable to the 100 to 300 kg of N $ha^{-1}$ $year^{-1}$ fixed by rhizobial legumes and actinorhizal non-legumes, but they are free, as it were. Little input is required by a manager except to ensure that a diversity of niches is maintained.

## Effects of the Environment on Nitrogen Fixation

Nitrogen fixation of both free-living and symbiotically-associated bacteria is profoundly affected by the environment. Effects depend upon the genetics and physiology of the host and the strain of the bacterial symbiont.

### Oxygen

Oxygen levels in the soil affect legume nodule activity. For soybeans, the optimum level of oxygen is $pO_2$ 0.5 atmospheres. Small

oxygen deficits depress nitrogen fixation, reducing respiration less. At higher levels of oxygen, nitrogen fixation is reduced without depressing respiration. A maximum for nodule respiration is at $pO_2$ 0.5 atmospheres, the same as for nitrogen fixation, suggesting that high respiration reduces the oxygen at the nitrogenase site and permits the reduction of nitrogen to ammonia. Usually soil oxygen levels are below the optimum for nitrogen fixation.

Carbon Dioxide

The effects of carbon dioxide ($CO_2$) are not too clear. Increasing the $CO_2$ levels in the atmosphere stimulates nitrogen fixation in some legumes by increasing photosynthesis. Whether or not $CO_2$ stimulates nitrogen fixation *per se* is a point in question.

Soil Water

Soil water profoundly affects the growth of the host and in legumes the water around the nodules affects the nitrogen-fixing activity within them. Reduction of water within a nodule inhibits respiration and nitrogen fixation.

Soil Acidity

In acid soils, calcium and molybdenum, which are essential to the symbiosis, are less available than in soils with high pH's and the nitrogen-fixing systems of legumes tend to be depressed. Acid soils do not often contain *Rhizobium*. There is less information about the *Frankia* symbiosis with non-legumes, but *Frankia* forms symbioses on a wide range of relatively acid forest soils and appears to be more tolerant of lower pH's.

Calcium

Temperate legumes have a fairly high calcium requirement, particularly for the period just following infection and nodule initiation. Calcium and pH interact; in acid soils more calcium is needed for rhizobial nodule formation on legumes than in soils with higher pH's. The situation for *Frankia* symbioses is not so clear. However, they appear to be relatively tolerant of lower pH's since they are active on relatively acid forest soils with low base contents.

Nitrogen

It is well known that nitrogen-fixing symbioses are sensitive to nitrogen levels. In legumes, the formation of rhizobial symbiosis is facilitated by a period of nitrogen hunger. When combined nitrogen (e.g. urea) is applied to a legume, root nodulation is inhibited, but the effect can be alleviated by increased photosynthesis or applications of sugar to the leaves. At very high plant calcium:nitrogen ratios, nitrogen fixation is inhibited. The hypothesis that the legumes internal carbon:nitrogen ratio governs nodulation and nitrogen fixation is still being examined. There are indications that combined nitrogen (e.g. $NO_3^-$) directly affects nitrogen fixation by inhibition of the production of root hairs (potential sites of rhizobial infection) and growth of the rhizobial infection thread. The effects of combined nitrogen on *Frankia* nodulation have been studied less intensively but appear to be similar.

Light

Nodulation is inhibited when roots are exposed to light (except for Trifolium subterraneum). When legume foliage is exposed to different colored light, nodulation is best in red light and poorest in blue. Nodulation is, however, inhibited by far-red light. Light filtering through green forest canopies contains more far-red light than red light, and shading might inhibit nodulation as well as reduce host plant photosynthesis. This is a point foresters should bear in mind when using nitrogen-fixing symbioses under tree canopies.

Temperature

Temperature affects nodule formation and functions at all stages. Nodulated plants occur in the Arctic and the tropics. In the Arctic, the symbioses are more adapted to cold conditions than those from further south. Root temperatures are more important than shoot temperatures in these relationships, although root and shoot cannot be considered in isolation. A forester contemplating using symbioses on northern, cold sites would do well, therefore, to remember the effects of host and bacterial symbiont provenances. The greatest development of nitrogen-fixing legumes is in the tropics, where warm climates favour these plants.

Most of the above comments apply to rhizobial symbioses. How far they apply to actinorrhizal symbioses is still a point in question, but there are likely to be many similarities.

For more information the reader is directed to the excellent reviews by Lie (1974) and Gibson (1977) upon which this synopsis is based.

Competition

In addition to the effects of environment, nodulation and nitrogen-fixing activity are affected by many competing higher plants and organisms in the soil. These other components of the forest biome not only compete for water, light and nutrients, but may release toxic compounds to inhibit their competitors by allelopathy. It is known that alder nodulation with actinorhizae (Frankia) can be inhibited by leaf leachates from balsam poplar (Populus balsamifera), the active agents possibly being polyphenols (Jobidon and Thibault, 1982). When this field is examined more closely, we are likely to find other examples of biochemical antogonism which will be of value to a forester using these symbioses as a forest management tool.

## The Genetics of Nitrogen Fixation

Nitrogen fixation is carried out my microbes which have no nuclei. Each bacterium possesses a circular, gene-bearing chromosome consisting of two intertwined strands of DNA (Deoxyribonucleic acid). Some bacteria possess one or more extra gene-bearing DNA rings, known as plasmids, which are much smaller (1%) than the main chromosome. These plasmids confer drug resistance, DNA transfer ability, substance utilization, etc.

The genes responsible for nitrogen fixation are called the nif cluster. On a Klebsiella chromosome, for example, this nif cluster is located between genes concerned with uptake of shikimic acid (shi) and synthesis of histidine (his). The nif cluster itself consists of two genes (called K and D) coded for producing the Mo-protein in nitrogenase, one gene (H) for the Fe-protein, and three regulatory genes E, A and L. In addition, there are F and B genes concerned with nitrogenase reduction and the formation of a part of the Mo-protein, respectively. The nif gene map for Klebsiella is: his.B. A. L. F. -- E. K. D. H. shi (Postgate, 1978).

Some plasmids confer to the bacterium the ability to transfer DNA to a new host by pseudo-sexual conjugation. This can be used to transfer active nif genes to bacterial recipients which do not have the ability to fix nitrogen. The nif genes can also be transferred to new bacterial hosts by bacterial viruses (bacteriophages) which can carry over DNA from a previous host to a new one. This situation lends itself to genetic engineering. The reader is referred to Postgate (1978) and Hollaender (1977) for more details. Plasmids have been recorded in Rhizobium many times and recently they have been recorded in Frankia (Normand et al., 1983).

In free-living organisms, the genetics are relatively simple, but in the case of nitrogen-fixing symbioses, they are much more complex; the genetics of both the bacterial symbiont and the host plant have to be taken into account. The bacterium and the host must be compatible (i.e. the host allows infection by the bacterium). If the bacterium is not autonomous for oxygen regulation at the nitrogenase site (e.g. Rhizobium), the host must possess the genes to produce heme compounds at the right place at the right time. In addition, the host must have the genes on its numerous chromosomes to permit it to assimilate, grow and compete. In the case of symbioses, genetic manipulation is complex, but there are many opportunities for progress, since both host and bacterium can be genetically selected and manipulated. For example, very little is known about the genetics of actinorhizal alders or the leguminous tree species, and alder selection and breeding is a wide-open field.

Scientists are searching to find ways of transferring the nitrogen-fixing capacity from one tree species to another, particularly to tree species of economic importance. Frankia symbioses are a great deal more promising than Rhizobium symbioses for interspecific transfer, since Frankia's host does not have to possess the complex genetic control of the heme compund to remove oxygen. Even in the case of Frankia, however, there are major obstacles to be overcome. Frankia strains from Elaeagnus do not appear to infect alders effectively, while strains of the actinomycete from Comptonia can infect alders. Even within one plant family, one genus will accept Frankia and another will not. In Betulaceae, for example, alders accept Frankia and form nitrogen-fixing symbioses but birches do not, even though the trees are similar in many ways.

The obstacle is the bacterum/plant incompatibility caused by an infectivity barrier, a physio-chemical characteristic that is genetically controlled. Before actinorhizal symbioses can be transferred to new higher plant hosts, the genetic constitution of either the Frankia, the tree host or both, must be manipulated to permit infection by Frankia and permit its nif genes to function within the host plant.

## Use of Nitrogen Fixation in Forestry

### Options and Constraints

A forester considering the use of nitrogen-fixing symbioses in his managed forest is faced by a large number of options and constraints. Legumes can be used to add nitrogen and organic matter to the soil and possibly provide a cash crop of vegetable oil (tree lupins) and even wood fibre if it is practicable to use arborescent legumes. Also, shrubby and arborescent non-legumes can be used to improve the soil and/or produce fibre. Some temperate non-legumes such as alders are very fast-growing.

A forester's selection of a technique is governed by biological constraints. Many legumes require a great deal of light to survive and nodulate, and may not perform well under forest canopies. Theoretically, nodulation of legumes could be inhibited by the high far-red component of light below tree canopies even if the plants grow. In addition, some shrubby and herbaceous legumes have vigorous growth and could compete with the trees for light, water and nutrients on better sites. In general, legumes do not perform well on many forest soils (Jorgensen, 1978).

Constraints are also associated with actinorhizal non-legumes. Comptonia, for example, is an aggressive pioneer and good soil improver on sandy soils that can overwhelm young trees. Also, it is known that alder litter tends to lower soil pH even on calcareous soils (Miller, 1982). This could have a considerable impact on other soil organisms and on alder nodulation. At pH's below 4.5, alder can develop endophytes that are relatively ineffective (Wheeler et al., 1981; Miller, 1982). The lowering of soil pH by alders even extends to the soil's B horizon, and in Douglas fir (Pseudotsuga menziesii) stands the base saturation tends to be low in both A and B horizons with lowered levels of calcium and magnesium (Franklin et al., 1968). Bollen et al., (1967) reported that nitrification is enhanced in alder litter despite the lowered pH.

Some non-fixers (e.g. Populus balsamifera) (Jobidon and Thibault, 1982) appear to inhibit alder germination and nitrogen-fixation and there are probably many other interactions also.

### Effects of Nitrogen-fixing Symbioses on Tree Growth and Soil in Forests

In spite of the constraints, there are many recorded cases where the use of nitrogen-fixing symbioses improves forest soil fertility and increases tree yields (e.g. Tarrant and Miller, 1963; Tarrant and Trappe, 1971; Miller and Murray, 1978; Finn, 1953; De Bell and Radwan, 1979; Haines and De Bell, 1979; Carmean et al., 1976). Nitrogen-fixing symbioses have been used frequently to rehabilitate degraded soils such as mine wastes (e.g. Plass, 1977).

A good example of the effects of alders was observed when Populus trichocarpa was planted with red alder (Alnus rubra) (De Bell and Radwan, 1979). After four years, the dry weight yields from the mixed poplar/alder stands were 35% or more greater than the yield of pure crops of either species, mainly due to the increased growth of the poplar. Soil nitrogen also increased. Another example is the effect of actinorhizal

autumn olive (Elaeagnus umbellata) in widely-spaced walnut (Juglans nigra) plantations. After nine years, the walnut height and diameter growths were 70% and 76% respectively greater than walnut in pure stands (Funk et al., 1979), in spite of the fact that the walnut had not responded to nitrogen fertilizers in previous tests.

Although the use of herbaceous legumes in forests raises many problems, Haines et al. (1978) reported that in the southern United States the height and volume growth of sycamore (Platanus) was increased two- or three-fold by establishing legumes such as clovers and vetches in the early stages of the plantations; Trifolium subterraneum and T. incarnatum provided good weed control. There is also evidence that in the tropics, legumes not only greatly increased the yield of rubber trees (Hevea) but helped to control root-rot diseases (e.g. Fomes). The reader is referred to the reviews by Haines and De Bell (1979), Miller (1982), Jorgensen (1978), and Zavitovski et al. (1979) for more details.

Although there is a great deal of scattered information about the use of nitrogen-fixing symbioses to improve forest soils and increase tree yield, many of the experiments were based on small plots, were site specific, and were sometimes not designed for the purpose. There is an urgent need for strictly controlled field tests of the effects of nitrogen-fixing symbioses on yield, soil fertility and host resistance to diseases in managed forests, and on the biology and ecology of the host plants involved.

Cultural Systems

The forester has many cultural options from which to choose. The one selected for any particular site will depend upon the climate, the type of soil, the type of microorganism/higher plant symbiosis being used, the shade and soil tolerance of the host plant, the form and aggressiveness of the latter, the characteristics of the main tree crop, and the rotation length.

The forester will need to decide whether or not nitrogen-fixing symbioses are wanted just to add nitrogen and organic matter or provide benefits such as additional fibre, grazing for wildlife, site stabilization and oil seed, or both of these. These decisions should be made in the context of the biology of both the symbioses and the main tree crop, interactions with the site, and the constraints discussed earlier.

Haines and De Bell (1979) reviewed the cultural systems available to the forester, and this section is based on their suggestions. The reader is referred to their paper for details.

A forester can (i) use rotation systems, (ii) establish mixed plantations containing trees, shrubs or herbs with nitrogen-fixing symbioses or (iii) grow the tree species with nitrogen-fixing symbioses as a commercial crop (e.g. alders) (Table 8).

Rotation systems include (a) green manuring and (b) alternation of trees without nitrogen-fixing symbioses and those with symbioses. If mixed plantations are considered, the forester can include nitrogen-fixing plants in the early stages of the plantation before the canopy closes, or,

Table 8: Different Cropping Systems for Use of Nitrogen-Fixing Symbioses

| | |
|---|---|
| 1. | ROTATION SYSTEMS |
| | (a) green-manuring with non-commercial, nitrogen-fixing plants |
| | (b) alternate crops of commercial species, one able, and the other unable, to fix nitrogen |
| 2. | MIXED SPECIES SYSTEMS |
| | (a) mixtures of two commercial species able to fix nitrogen |
| | (b) non-commercial, nitrogen-fixing species in the understorey for most of the rotation |
| | (c) non-commercial, nitrogen-fixing species in the understorey only until the canopy closes |
| 3. | NITROGEN-FIXING TREES AS THE PRINCIPAL CROP |

(from: Haines and De Bell, 1979)

if the nitrogen-fixing plants can ccapete with the main crop or tolerate shading, they can be kept in the stand throughout the rotation. A forester can, of course, grow nitrogen-fixing trees (e.g. Robinia and Alnus) as a main crop if the sites are suitable and there is a market for the product. Shrubby and herbaceous legumes have potential value for rotations including green manuring and growing between the trees before the canopy closes, particularly in the intensive poplar, sycamore, etc. systems on short (<12 year) rotations. More testing is needed, however, to discover the extent to which the legumes reduce short-term tree growth by competing for water, light, and nutrients.

If necessary, a nitrogen-fixing green crop can be used (such as tree lupins) that yields a commercial product (e.g. oil seed). Care will be necessary to ensure that the legume is not too aggressive. If the legumes are grown in rotation, they can be sprayed with herbicides or turned into the soil to increase the nitrogen and organic matter content.

The use of legumes in those systems, either as a green rotation crop or betwen the tree rows is limited by the fact that many legumes do not grow well or nodulate effectively on the less fertile forest soils. The opportunities seem to be greatest on relatively fertile agroforestry sites and better hardwood soils (provided the nitrogen content is not so high as to inhibit nodulation). However, some legumes, such as lupins and vetches, are pioneer plants and can grow on very poor soils, and may prove to be valuable in forests.

Commercially valuable tree species can be grown on alternate rotations with non-fixing trees, to improve the soil and provide fibre. This overcomes the disadvantage of a green manure rotation which removes the site from fibre production for a period. However, the green manuring period need only be short (one to three years) if herbaceous legumes are used as the green crop.

Non-legumes such as Alnus, Myrica, and Comptonia are suitable for the poorer soils and can be planted on adverse sites to prepare the soil for later planting with commercial species. This has been done on coal spoil wastes.

The arborescent non-legumes, such as alder, are suitable for interplanting or alternating as a secondary commercial species. Although alders are pioneers and reproduce vigorously in natural situations, some attempts to establish them on forest sites have met with difficulties. It is clear that there is still a great deal to be learned about propagation, establishment, weed control, and maintenance of alder plantations, as well as selection of sites.

## Economics

A forester will choose the cultural option that best achieves his management objectives (i.e. fibre production) with the minimum of cost. Beuter (1979) discussed the economics of fertilization and nitrogen-fixing symbioses in forestry in relation to the Entropy Law, and concluded that "wide-spread gains in long-term harvests are more likely to come from management designed to enhance nitrogen-fixation than from artificial fertilization". The economics of mixing red alder with Douglas fir or applying fertilizer were examined.

In Douglas fir and Douglas fir/alder plantations, it was found that the use of artificial fertilizer ranked first economically, a small margin better than the alder option. However, in this experiment the cost of planting 1,210 alder per acre (2,990 alder $ha^{-1}$) heavily weighted the alder option. If fewer alder gave a comparable growth benefit the nitrogen fixation option would look much more favourable (Atkinson and Hamilton, 1978). The number of alders needed per unit of land area to confer significant soil and growth benefits is not yet known.

Comparisons of the economics of using alder and fertilizers are usually based on the value of the fibre produced in the short term (i.e. one rotation). It is also frequently said that the feasibility of using nitrogen-fixing systems in forestry depends upon the costs and availability of fertilizers, and that when fertilizers are cheap and plentiful interest in nitrogen fixation as a forest management tool will wane as it did in agriculture. Since they abandoned legume rotations, farmers are learning that this was short-sighted. As described earlier, chemical fertilizers can restore deficient elements but cannot restore soil organic matter which is vital to long-term site productivity. In addition, chemical fertilizers can upset nutrient balance, adversely affect soil organisms, and pollute water resources. Any cost/benefit analysis should consider these facts. A forester may have to consider aiming at an optimum yield per unit investment that permits longterm maintenance of site productivity, rather than striving for maximum yield which is the current trend.

There is still a great deal to learn about the genetics, biology, ecology and culture of nitrogen-fixing systems in forests before they can be used effectively in forestry. For further information, the reader is referred to the reviews in Gordon et al. (1979).

## MYCORRHIZAL SYMBIOSES

Mycorrhizae are symbiotic associations between fungi and the roots of higher plants. The fungi are not pathogenic (or are only weakly parasitic) to the plant and exist in the root cortices in various degrees of intimacy with the root tissues.

The fungus and the plant confer benefits to each other which enable both to survive. The fungus is provided with a place to live and with a supply of food and energy by the higher plant. The host plant benefits from the fact that mycorrhizal roots assist in water and nutrient uptake, provide reserves for times of stress, and protect roots against diseases. the mycorrhizae also play a role in the ecosystem by interacting with other soil microorganisms and processes.

### Types of Mycorrhizae

Mycorrhizae fall into three classes namely, Ectomycorrhizae, Endomycorrhizae, and Ectendomycorrhizae. The latter has features of both the other two. The fungi associated with these classes are given in Table 9.

Ectomycorrhizae occur on only about 5% of vasicular plants, but these include many conifers and some hardwoods of commercial importance. The fungi involved are mainly Basidiomycetes, Ascomycetes and Gasteromycetes, and over 2000 of these fungi are potential symbionts.

Endomycorrhizae include three types: the Vesicular Arbuscular (VA) (sometimes called phycomycetous mycorrhizae), the Ericoid, and the Orchidaceous. The Va type, the aseptate mycelium type, is important in forestry and occurs on many angiosperm trees and shrubs. The Ericoid type occurs on species such as Calluna, Erica, Vaccinium and Rhododendron. The Orchidaceous type occurs on orchids and gentians.

Ectendomycorrhizae occur on some woody plants, including the conifers. These include the Arbutoid types which occur on Arbutus, Pyrola and Arctostaphylos (Trappe, 1977; Mexal, 1980; Linderman, 1978) and the nursery ubiquitous E-strain described by Wilcox (1982). The fungal symbionts are not well understood, but Fungi Imperfecti and Complexipes spp. appear to be involved (Table 9).

This report will focus on VA endomycorrhizae and ectomycorrhizae.

### Characteristics

Endomycorrhizae grow inside and between the cells of the root's cortex, penetrating deeply into the cells and tissues without forming an external mantle of fungal hyphae over the root surface. Vesicular arbuscular endomycorrhizae form vesicles and arbuscules within the cells and many form abundant, resting chlamydospores at the root surface. The fungal hyphae can be septate or nonseptate (aseptate). Ectomycorrhizae typically grow in the network of spaces between the cells in the outermost tissues of the root cortex, and in most cases, form a thick mantle of fungal tissue over the tip of a feeder root with hyphae extending into the soil. The fungal network in the root cortex is called the Hartig net. Ectendomycorrhizae occupy spaces between the cells of the root cortex, as

Table 9: Type of Mycorrhizae

| | Host Plant | Symbiotic Fungus |
|---|---|---|
| **ENDOMYCORRHIZAE** | | |
| i Vesicular Arbuscular (VA) (Phycomycetous) | Bryophytes<br>Pteridophytes;<br>Gymnosperms except the Pinaceae;<br>Angiosperms (about 90-95% of vascular plants) | Endogonaceae (excluding Endogone)<br>Gigaspora;<br>Glomus;<br>Acaulospora;<br>Sclerocystis; etc<br>(Non-septate hyphae) |
| ii Ericoid | Calluna; Erica; Rhododendron; Vaccinium etc. | Pezizella ericeae<br>Clavaria (?)<br>Not well known<br>(Septate hyphae) |
| iii Orchidaceous | Orchids;<br>Gentians | Basidiomycetes;<br>Rhizoctonia;<br>Tulasnella;<br>Thanoptophorus;<br>Ceratobasidium |
| **ECTOMYCORRHIZAE** | Only on woody plants e.g. Tiliaceae; Pinaceae; Betulaceae; Fagaceae; Juglandaceae; Salicaceae; Ulmaceae; Rosaceae; Leguminoceae | Ascomycetes;<br>Gasteromycetes;<br>Basidiomycetes |
| **ECTENDOMYCORRHIZAE** | | |
| i Common type | Woody plants;<br>Pinaceae | Fungi Imperfecti<br>Complexipes moniliformi;<br>Not well known |
| ii Arbutoid | Pyrola, Arbutus Arctostaphylos | Cortinarius spp.<br>Not well known |

(revised from: Gerdemann, 1968)

in ectomycorrhizae, and many even form a loose fungal mantle. However, some hyphae may penetrate the cortical cells like endomycorrhizae.

Mycorrhizae Formation

The mycelium of a potential VA endomycorrhizal symbiont often colonizes the plants via the root hairs. Before doing so, the mycelium, from either resting hyphae or a germinated spore, must penetrate the rhizosphere where carbohydrate levels may be high. However, so are the populations of competing microorganisms. Once the mycelium reaches the root hair or epidermal cells, the hyphae can invade the host's root tissue provided the fungus and the host are compatible.

The VA endomycorrhizal fungi produce thick-walled resting spores which germinate and send hyphae into the cells of the host's epidermis and cortex and fill them with branched, hyphae known as arbuscules. The establishment of the arbuscules, the centre for nutrients taken up by the hyphae, establishes a situation of mutual benefit to invader and host plant. Eventually, the arbuscule breaks down, releasing nutrients which the cell digests and uses, thereafter returning to normal. Other cells are then invaded by arbuscule-producing hyphae.

The fungus sometimes produces large, swollen vesicles within the cells; the function of vesicles is not fully understood, but they often act as resting chlamydospores.

The fungal symbiont sends hyphae into the soil which ultimately form sporocarps, or isolated spores, that can begin the root infection cycle again.

In the case of ectomycorrhizal fungi, there are usually many hyphae, spores, sclerotia, mycelial strands, etc. in the soil that can germinate, penetrate the rhizosphere and infect the roots. Once within the host roots, the fungus invades the spaces between the cells of the root's cortex, establishing a network (the Hartig net) by which water and nutrients can be exchanged. Often a mantle of hyphae grows over the tip of the root, and hyphae extend into the soil. The strands of hyphae can extend great distances (several metres) through the soil and produce the familiar fruiting bodies of mushrooms.

In both these cases, the fungi act in similar ways to fungal pathogens in their infection and invasion, but instead of destroying the host plants' cells, they provide them with beneficial nutrients in a state of symbiotic balance. At low temperatures, a mycorrhizal symbiont can become weakly parasitic on the host (Furlan and Fortin, 1973).

Mycorrhizae in Trees and Shrubs

Endomycorrhizae are widely distributed in trees and shrubs throughout the world, although the endotrophs have not been studied in detail in the gymnosperms. They occur in pioneer species of the north (eg. Dryas), on a variety of sites in temperate forests (Alnus, Salix, Acer, Juniperus, Juglans) and in the tropics (Avocado, Cunninghamia, Podocarpus, etc.) (Table 10). They occur in many gymnosperms except those of the Pinaceae which are ectomycorrhizal or sometimes ectendomycorrhizal (Table 11). Endomycorrhizae also form on trees and shrubs with rhizobial or actinorhizal (Frankia) nitrogen-fixing symbioses. Alnus can have the triple endomycorrhizal, ectomycorrhizal and actinorhizal (Frankia) symbioses on the same plant.

Table 10: Genera of Forest Trees and Shrubs with Endomycorrhizae [2,3]

| Genus | Gymnosperm (G) or Angiosperm (A) | Nitrogen-Fixing Symbioses | Source |
|---|---|---|---|
| Acer** | A | - | Wilcox et al. (1981) |
| Agathis | G | - | Harley (1969) |
| Alnus** | A | ACN[1] | Walker and Bartlett (1981); Rose (1980) |
| Araucaria | G | - | Harley (1969) |
| Avocado | A | - | Harley (1969) |
| Biota | G | - | Harley (1969) |
| Caragana** (?) | A | RN[1] | |
| Carica | A | - | Maronek et al. (1981) |
| Casuarina | A | ACN[1] | Rose (1980) |
| Ceanothus | A | ACN[1] | Rose (1980); Rose and Youngberg (1981) |
| Cercocarpus | A | ACN[1] | Rose (1980) |
| Cephalotaxus | G | - | Harley (1969) |
| Chamaecyparis | G | - | Harley (1969) |
| Citrus | A | - | Harley (1969) |
| Comptonia** | A | ACN[1] | |
| Cryptomeria* | G | - | Harley (1969) |
| Cunninghamia | G | - | Harley (1969) |
| Cupressus | G | - | Harley (1969) |
| Dryas | A | ACN[1] | Rose (1980) |
| Elaeagnus** | A | ACN[1] | Riffle (1977, 1980) |
| Fraxinus** | A | - | Pope et al. (1981); Hexal (1980) |
| Ginkgo | G | - | Harley (1969) |
| Glyptostrobus | G | - | Harley (1969) |
| Hevea | A | - | Harley (1969) |
| Hippophae** | | ACN[1] | Rose (1980);Rose and Trappe (1980) |
| Juglans* | A | - | Ponder (1981) |
| Juniperus | G | - | Harley (1969) |
| Libocedrus | G | - | Harley (1969) |
| Liquidambar | A | - | Davis et al. (1981) |
| Liriodendron | A | - | Pope et al. (1981) |
| Magnolia | A | - | Maronek et al. (1981) |
| Malus | A | - | Plenchette et al. (1981) |
| Metasequoia | G | - | Harley (1969) |
| Paulownia | A | - | Beckjord (1981) |
| Platanus** | A | - | Pope et al. (1981) |
| Podocarpus | G | - | Harley (1969) |
| Populus** | A | - | Vozzo and Macskaylo (1964) |
| Purshia* | A | ACN[1] | Rose (1980) |
| Quercus* | A | - | Marx (1977); Grand (1969) |
| Robinia** | A | RN[1] | Daft et al. (1975) |
| Salix** | A | - | Fontana (1962) |
| Sciadopitys | G | - | Harley (1969) |
| Sequoia** | G | - | Harley (1969) |
| Sequoiadendron | G | - | Harley (1969) |
| Shepherdia** | A | ACN | Riffle (1980; Rose (1980) |
| Taiwania | G | - | Harley (1969) |
| Taxodium | G | - | Harley (1969) |
| Taxus | G | - | Harley (1969) |
| Thuja* | G | - | Harley (1969) |
| Thujopsis | G | - | Harley (1969) |
| Torreya | G | - | Harley (1969) |
| Ulmus | A | - | Hexal (1980) |

Notes: [1] ACN = Actinorhizal Symbiosis (Frankia)
RN = Rhizobium Symbiosis

[2] Some genera with endomycorrhizae may contain ectomycorrhizae also

* Genera of potential value as soil improvers or fibre producers in general forestry in temperate to south temperate zones

** Of potential value as soil improvers or fibre producers in intensive biomass production

[3] The list is not complete. Many more angiosperms are likely to have endomycorrhizae, and gymnosperm endomycorrhizae have not been studied in detail

Table 11: Tree Genera with Ectomycorrhizal Symbioses

(1) GYMNOSPERMAE

(1a) Pinaceae
- Abies**
- Cathaya
- Cedrus*
- Keteleeria
- Larix**
- Picea*
- Pinus**
- Pseudolarix
- Pseudotsuga
- Tsuga*

(1b) Cupressaceae
- Cupressus*
- Juniperus

(2) ANGIOSPERMAE

(2a) Juglandales
- Juglandaceae
  - Carya
  - Juglans

(2b) Salicales
- Salicaceae
  - Populus**
  - Betula**
  - Carpinus
  - Corylus
  - Ostrya
  - Ostryopsis

(2c) Fagaceae
- Castanea**
- Castanopsis
- Fagus*
- Lithocarpus
- Nothofagus**
- Pasania
- Quercus**
- Trigonobalanus

(2d) Urticales
- Ulmaceae
  - Ulmus

(2e) Rosales
- Rosaceae
  - Crataegus
  - Malus
  - Pyrus
  - Sorbus

(2f) Leguminosae RH[1]
- Afzelia RH
- Anthonotha RH
- Brachystegia RH
- Gilbertiodendron RH
- Julbernardia RH
- Monopetalanthus RH
- Paramacrolobium RH

(2g) Sapindales
- Sapindaceae
  - Allophylus
- Aceraceae
  - Acer**

(2h) Malvales
- Tiliaceae
  - Tilia

(2i) Myrtiflorae
- Myrtaceae
  - Eucalyptus**

(2j) Ericales
- Ericaceae
  - Arbutus

Notes: * Includes important commercial trees
** Includes commercial tree species listed as promising for intensive forestry (Lavoie and Vallee (1981) (Appendix I)
1 RH = Rhizobium symbiosis

(from: Meyer, 1973)

Endomycorrhizae occur on trees and shrubs of potential value as soil improvers (Alnus, Caragana, Elaeagnus) and fibre producers in general forestry (Cryptomeria, Juglans, Thuja, etc.). These endophytes also occur on trees and shrubs of potential value in forests intensively managed for biomass production for energy and other purposes (eg. Platanus, Populus, Robinia, Salix, Sequoia, Ulmus, etc.).

Although ectomycorrhizae are less common than endomycorrhizae, they are distributed throughout the world, and occur from the north temperate zone to the tropics. Ectomycorrhizae occur on all the Pinaceae, including such important tree genera as Abies, Cedrus, Larix, Picea, Pinus, Pseudotsuga, and Tsuga, and in many angiosperms (Alnus, Acer, Betula, Salix, Populus, Castanea, Fagus, Quercus, Eucalyptus, Tilia, etc.). Many of these are of value in intensive forestry (Appendix I). These mycorrhizae also occur in arborescent tropical legumes such as Afzelia and Anthonotha, which have Rhizobium symbioses and provide an example of a double (ectomycorrhizae/Rhizobium) symbiosis.

In Appendix I, the types of mycorrhizae associated with different tree species are indicated as they are given in literature references. Many of the references refer only to genera, but in some genera, for example Pinus, Salix, and Populus, it is very likely that all species are mycorrhizal, even through some may not have been examined. The main effort in research on mycorrhizae has been on agricultural and horticultural crops.

Although mycorrhizae are very widespread, some plant families are rarely mycorrhizal or non-mycorrhizal. These include the Cruciferae, Cyperaceae, Juncaceae, Urticaceae, Fumariaceae, Caryophyllaceae, Portulacaceae, Amaranthaceae, Chenopodaceae and Polygonaceae (Gordemann, 1968). Few crucifers have been observed with mycorrhizae. Some of the higher plants which form mycorrhizae seem to be obligate symbionts, at least under natural field conditions (i.e. they cannot survive without them). In mycorrhizal plants the entire root system, or only part of it, can be infected by the fungus.

## The Benefits of Mycorrhizae to Higher Plants

Many mycorrhizae develop best when the higher plants are under some kind of stress, particularly low nitrogen levels, but some mycorrhizae develop well in non-stress situations. In agriculture, these stresses can be controlled to some extent by irrigation and fertilization. In forestry, irrigation is not possible except in nurseries and small plots; extensive fertilization is very costly in terms of both energy and money and is seldom practicable. In the forest, therefore, most of the trees have to make do with what they have.

Earlier in this report the possible benefits of using nitrogen-fixing symbioses to maintain or upgrade forest soil nitrogen and organic matter were discussed. Mycorrhizae can also play a role by enabling the trees to make best use of what is present in the soil, and allowing them to withstand the stresses of water, disease, etc. that occur in the forest. In general, mycorrhizae benefit higher plants by (Table 12a):

(a) improving water uptake, a point still being studied; plants with mycorrhizae appear to be able to stand drought better than those without

Table 12a: Positive Effects of Mycorhizae

| Type of Mycorrhizae | Physiological Effects |
|---|---|
| **A. ENDOMYCORRHIZAE** | |
| i Vesicular-Arbuscular (VA) | a) better access to non-mobile elements such as P, Zn and Cu<br>b) resistance to diseases<br>c) better water uptake<br>d) provide carbohydrate and lipid (?) sinks |
| ii Ericoid | a) enables the plants to take up nitrogen in an organic form<br>b) provides a nitrogen reserve<br>c) excludes toxic elements |
| iii Orchidaceous | a) permits development<br>b) protects against diseases |
| | **Positive Effects** |
| **B. ECTOMYCORRHIZAE** | |
| | a) improved uptake of $PO_4^{--}$, $Ca^{++}$, $K^+$, $Cl^-$, $SO_4^-$, $Na^+$, $NO_3$, $NH_4^+$, $Mg^{++}$, $Fe^{++}$, $Zn^{++}$ ions<br>b) greater availability of nutrients from a large soil volume<br>c) presence of phosphatase and/or nitrogen reductase (in some ectomycorrhizae)<br>d) secretion of growth-regulating substances<br>e) provides a carbohydrate and lipid (?) sink<br>f) production of volatile substances and antibiotics which exert selective pressures on nearby plants and organisms<br>g) utilizes exuded root carbohydrates that would otherwise attract pathogens<br>h) greater resistance to some soil pathogens by possessing a fungal mantle<br>i) detoxification of soil phytotoxin by some mycorrhizae<br>j) improve soil texture by binding soil aggregates |
| **C. ECTENDOMYCORRHIZAE** | a) not well-known, but probably similar to ectomycorrhizae |

(based on: Fortin in 1982 (mimeo); Langlois and Fortin, 1981)

(b) improving nutrient uptake, particularly on poor soils where the nutrients are present in relatively unavailable forms (eg. phosphorus)

(c) permitting plants of the Ericaceae to take up nitrogen in organic forms

(d) modifying the host plant's root physiology, resulting in root proliferation, etc; application of mycorrhizae are known to enhance the rooting of tree and shrub cuttings

(e) providing carbohydrate and lipid sinks for use when needed

(f) protecting the roots from soil-borne fungal pathogens

It has also been suggested that mycorrhizae, which have the ability to take up metals etc., can be used to detoxify soils (Linderman, 1978). There are still many effects not yet confirmed. In some plant species, mycorrhizae appear necessary for their survival.

Negative effects of mycorrhizae are listed in Table 12b.

Carbohydrates and Lipids

The fungus symbiont requires carbohydrate from the host for energy in mycorrhizal symbiosis. It appears that the amount of soluble carbohydrates in the host influences the degree of mycorrhizal development (Maronek et al., 1981). Ectomycorrhizal fungi appear to be capable of converting soluble sugars into storage compounds such as glycogen, trehalose and mannitol, which are fungal carbohydrates not readily metabolized by the host. It has been suggested that the fungus provides a carbohydrate sink which may exist at certain seasons and meet the needs of both itself and the host. Storage carbohydrates such as trehalose and mannitol do not seem to occur in endomycorrhizae. In some agricultural plants there are more lipids in endomycorrhizal roots than non-mycorrhizal roots. Oil droplets occur in hyphae as well as in vesicles and chlamydospores. The fungus may be acting as an alternative sink for host photosynthates, and the lipids may be an important storage reserve. However, these are hypotheses and require confirmation (Maronek et al., 1981; Ho and Trappe, 1975; Cox et al., 1975).

Nutrient Uptake

There is abundant evidence that mycorrhizae are advantageous to crops grown on relatively poor soils, including disturbed, reclaimed landscapes associated with industry.

As early as 1937, it was known that white pine (Pinus strobus) seedlings with ectomycorrhizae weighed more and had higher nitrogen and phosphorus contents than non-mycorrhizal plants. This also occurs in other woody plants and herbs (Maronek et al., 1981; Hatch, 1937; Meyer, 1974; Trappe, 1977).

Endomycorrhizae and ectomycorrhizae increase the uptake of phosphorus by many plants on infertile soils. Tracer experiments have shown that the ectomycorrhizal mantle of fungus accumulates and stores

Table 12b: Negative Effects of Mycorrhizae

1. Short-term utilization of carbohydrates, etc. that could be used by the host for other purposes. (In the long-term, some carbohydrates in the mycorrhizal sink are used by the host.)

2. Unless care is taken in fungus selection, mycorrhizal fungi or their close relatives might become too aggressive and eliminate plants by excessive toxic and antibiotic secretions (eg. Thelephora laciniata (Kavina, 1923; Iyer et al., 1980).

3. Mycorrhizae appear to be able to retard the processes of soil organic matter mineralization, ammonification and nitrification, locking up nitrogen and other elements. This should respond to silvicultural treatments and could be beneficial as a sort of nitrification control (Gadgil and Gadgil, 1974).

4. In some circumstances, particularly on fertile soils with high phosphorus levels, mycorrhizae may reduce plant growth by using carboydrate which could be used by the fungus.

Note: Most of these negative effects could be reduced by selection of suitable symbionts for the site, crop, and soil management technique.

phosphorus, and a phosphorus deficiency in the plant can release phosphorus from the mantle. Whether or not ectomycorrhizae can store other nutrients is not so clear. Many of the good responses of plants to ectomycorrhizae can be attributed to improved phosphorus nutrition. The same applies to endomycorrhizae, and horticultural plants, including orange trees (Citrus spp.), which increased their phosphorus content when inoculated with these endophytes.

The relationship between formation of endo- and ecto-mycorrhizae and uptake of nitrogen and potassium is conflicting; sometimes mycorrhization helps, and sometimes it does not. There is also evidence that mycorrhizal plants have better uptake of zinc, calcium, copper, and magnesium, but the effect is variable and seems to be influenced by individual host-fungus interaction and soil fertility.

The phosphorus influx rate can be increased up to sixteen times. Clearly the mycorrhizal hyphae extending from the roots greatly increase effective root surface area and increase phosphorus uptake opportunities, particularly in the extensive mats of ectomycorrhizae in forest soils. In addition, mycorrhizae increases the effective life of feeder roots (Riffle and Boosalis, 1979). It is possible that mycorrhizal hyphae, in association with the soil bacterial flora, are able to make soluble the relatively inert forms of phosphorus not available to plant roots. It has been reported that pine and beech mycorrhizae can absorb phosphorus from compounds containing the element in a form unavailable to plant roots with enzymes on the mycorrhizal surfaces (Maronek et al., 1981). However, the way in which mycorrhizae aids phosphorus uptake is not clearly understood.

Although there have been reports of mycorrhizae with the ability to "fix" atmospheric nitrogen, this has never been confirmed. It is known, however, that mycorrhizae occur on nodulated plants and can stimulate nodulation. Mycorrhizal plants improve phosphorus uptake, and in some cases, zinc and copper uptake. Phosphorus is vital to the nitrogen-fixing process via the energy (ATP → ADP) supply; zinc and copper are known to affect nodulation. There are reports that after a nitrogen-fixing bacterium was inserted into an ectomycorrhizal mycelium, the association fixed nitrogen (Giles and Whitehead, 1976). However, this has not been confirmed either.

### Water

There are many reports that mycorrhizal plants exhibit better drought tolerance than non-mycorrhizal plants. For example, Monterey pine inoculated with Rhizopogon luteolus survived a dry summer better than plants without mycorrhizae, and oak seedlings without mycorrhizae wilted sooner than the mycorrhizal plants. The mycorrhizal fungi themselves vary a great deal in tolerance to soil water levels. In general, wet soils inhibit formation of mycorrhizae, but some mycorrhizae, such as Hymenogaster alnicola Vitr. and Lactarius obscuratus Pers. which form alder mycorrhizae, perform well on wet sites. The mycorrhizal fungus Cenococcum geophilum was more tolerant of very dry soil conditions than Suillus leteus Gray (Maronek et al., 1981).

### Disease Resistance

Theoretically, ectomycorrhizae should be able to protect roots against soil-borne pathogens because they utilize the roots surplus

carbohydrates which could attract pathogens and they provide the protection of the mantle of fungal hyphae outside the roots.

Although it appears that mycorrhizae protect plants from diseases, the evidence for endomycorrhizae is conflicting. These symbioses can increase, decrease, or have no affect on the severity of diseases depending upon the host plant species and the types of mycorrhizal fungi and pathogens involved (Dehne, 1981).

In ectomycorrhizae the matter is a little clearer. It was found that in the shortleaf pine (P. echinata Mill.) all non-mycorrhizal roots were infected by the pathogen Phytopthora cinnamomi (Rands), but those with mantles of the mycorrhizal fungi Thelephora and Pisolithus were resistant (Marx, 1970). A few roots with gaps in the fungal mantles were infected (Marx, 1970; Marx and Davey, 1969b). Since all the roots of a mycorrhizal plant may not be infected or have well-developed mantles, the resistance to attack tends to be partial. There is some evidence that the Hartig net, typical of ecomycorrhizae, also acts as a barrier to infection, a second line of defence.

Endomycorrhizae do not have mantles and it is possible that they confer resistance by producing antibiotics antagonistic to fungi, bacteria and viruses. Ectomycorrhizal fungi are known to produce antibiotics (for example, diatretyne nitrile in the Leucopaxillus - Pinus echinata symbiosis) but this hypothesis requires further examination (Maronek et al., 1981).

The mycorrhizal fungi strongly affect the micoorganisms in the rhizosphere and could protect plants from pathogens by this attribute. Populations of bacteria, fungi, etc. differ around mycorrhizal and non-mycorrhizal roots. Pythium and Fusarium, pathogens that occur frequently in the non-mycorrhizal rhizosphere, are absent from the mycorrhizal rhizosphere. Some bacteria, often with complex nutrient requirements, only occur in the mycorrhizal rhizosphere (the mycorrhizosphere). Actinomycetes and ammonifying bacteria are also more common around mycorrhizal roots than around non-mycorrhizal roots. This rhizosphere effect may be one reason the endomycorrhizae, which have no protective fungal mantle, appear to be able to confer disease resistance on hosts (Maronek et al., 1981).

It is known that mycorrhizae induce changes in the morphology and chemistry of the host plants. It is possible that the symbiotic fungi stimulate the host to produce compounds that inhibit pathogen invasion. Again this is hypothetical and requires investigation (Marx, 1972; Wilhelm, 1973; Becker, 1976).

Although it appears that mycorrhizae protect the plants from infection by soil-borne pathogens, the available information is conflicting and needs further investigation.

Soil Acidity (pH)

On some sites, such as coal spoil heaps, the soil is very acidic (pH 3.0 to 3.8) and there may be high levels of toxic aluminum, manganese, copper and iron than can adversely affect plant growth. On very acid spoil heaps, Marx (1975) found that by using a selected mycorrhizal fungal

symbiont (Pisolithus tinctorius) better survival of Pinus virginiana Mill. and P. resinosa Ait. was obtained than with Thelephora terrestris.

Although it is known that some ectomycorrhizal fungi are very sensitive to heavy metals such as copper and arsenic (Harris and Jurgensen, 1977), some fungi are known to have high heavy metal tolerances and if these form mycorrhizae, they may be of use in detoxifying soils. This is an area requiring research. Many pesticides contain heavy metals and could adversely affect sensitive mycorrhizal symbionts of trees and other crops when used, a factor to be remembered when planning pest control operations.

Plant Growth Responses to Mycorrhizae

The effects of mycorrhizae on tree growth are the net results of the positive and negative effects of the fungus and host on each other. Like any other symbiosis, the mycorrhizae contribute to their partners and obtain energy and protection in exchange.

Mycorrhizal fungi vary considerably in their effect on tree growth, and need to be selected with care to obtain the maximum benefits for the particular tree and site involved.

The demands by the fungus for carbohydrate from the photosynthesizing host is a drain on the host, since carbohydrate energy is being diverted from other plant functions. The fact that these carbohydrates may be taken out of circulation only for a while, and stored as a sink that can be tapped by the host plant in times of stress, is a long-term advantage but may be a short-term disadvantage. Whether or not this short-term energy drain is a disadvantage depends on the fungi involved and the fungus-host-site relationships. There are examples of ectomycorrhizae inoculation depressing tree seedling growth. For example, the growth of Pinus ponderosa Laws. seedlings was depressed up to six months after inoculation by all mycorrhizal species tested by Trappe (1977) while the growth of Tsuga heterophylla (Raf.) Sarg. was improved by inoculation, with the fungus Pisolithus tenctorius having the best effect. Carbohydrate energy drain by the mycorrhizal fungi may well be one of the causes of growth reduction.

The ratio between roots and shoots of conifer seedlings can be affected by inoculation with Pisolithus tinctorius or Thelephora terrestris. This could be important in seedling establishment (Mexal, 1980).

Pronounced tree growth responses have been obtained by ectomycorrhizal inoculation of seedlings of P. taeda (Marx et al., 1977), P. radiata, and P. elliottii (Marx and Artman, 1978; Lamb and Richards, 1974).

There is also evidence that endomycorrhizae improve the growth and survival of hardwoods such as Liquidambar and Malus (Plenchette et al., 1981; Kormanik et al., 1977).

These growth increases, except in Malus, occur in either fumigated or non-fumigated soils. The practice of nursery soil fumigation to reduce incidence of pests and diseases can eliminate mycorrhizal fungi from the

soil; without plant or soil inoculation no mycorrhizae could develop. Positive growth responses with mycorrhizae are to be expected on non-fumigated sites, and in sterile container media. The same applies to agriculture, particularly in the United States, where huge areas are fumigated with toxic fungicides, etc. which destroy both beneficial or even essential microorganisms as well as the pathogens.

There is evidence of host genotype and fungus interaction, for example, in Pinus elliottii var. elliottii (Marx and Bryan, 1971) as well as host and fungus genotype interactions, for example in Betula verrucosa and Pinus radiata (Mason, 1975; Theodoru and Bowen, 1970). These illustrate the care needed in selection of symbionts for particular hosts.

The site and fungus interactions are also important and it was pointed out earlier in that mycorrhizal fungi differ in their response to soil pH, drought, heavy metals, etc. The forester may have to decide whether a symbiont should be selected for best growth response, site tolerance or a compromise of both. A good example is the drought resistant Cenococcum geophilum Fr. which is valuable for tree survival on very dry sites, but relatively inefficient for promoting seedling growth. If survival is the main concern, and rapid growth secondary, this fungus could well be the best option (Mexal and Reid, 1973; Mexal, 1980).

The improvement in tree growth described above refers to seedlings. Little is known about the response of the plants in plantations to mycorrhizae, and what is known tends to be confusing. Experiments have shown that the survival and growth of mycorrhizal seedlings is better than non-mycorrhizal seedlings when established in field conditions (eg. Marx et al., 1977; Theodoru and Bowen, 1970). This fact is clear, but the interpretation of causal effects is more clouded.

The size of tree seedlings of transplants can have a profound and long-lasting effect which tends to increase with time (Sweet and Wareing, 1966). A seedling that is initially large has a long-term advantage over smaller seedlings. In general, mycorrhizal tree seedlings raised in a nursery or in containers are larger than non-mycorrhizal seedlings. When comparative experiments are established in the forest, the larger mycorrhizal trees have an advantage over the smaller non-mycorrhizal trees. The subsequent better growth of the mycorrhizal trees might theoretically be due to seedling size rather than the benefits conferred to the trees by fungal symbionts per se. The effect of mycorrhizae on nursery seedling size is undeniable and only the subsequent direct effects of mycorrhizae in the field is in question, remembering that some of the host-fungus symbioses are obligatory for survival. These research problems can be solved by field experiments designed to eliminate the effect of seedling size by covariance analysis.

It is likely that in many cases, the selected mycorrhizal symbionts will ultimately be displaced by the wild population of fungi in forest conditions, unless the selected mycorrhizal fungus is aggressive. Even if the beneficial effects of a selected symbiont last for only a relatively short time in the life of the trees (five years), these short-term effects will profoundly affect future yield and survival. If direct long-term benefits are conferred to trees by mycorrhizal symbionts, this will be a bonus. The fungal species that are efficient and competitive on young trees are not necessarily as effective on mature trees.

Other Effects

There are many other effects, some of which are not clearly understood. In some circumstances, particularly when phosphorus levels are high, the mycorrhizae uses energy to take up more phosphorus (luxury consumption) than the host plant needs, and tree growth can diminish. It is not known whether this is due to toxic levels of phosphorus or excessive energy drain.

Mycorrhizae also appear to affect soil structure, and it has been suggested that soil aggregates greater than 2 mm in size depend upon binding by mycorrhizal hyphae. Hyphae of VA endomycorrhizae outside the root (extramatrical hyphae) are known to play a role in binding soil grains on sandy soils and helping to stabilize sand dunes (Smith, 1980).

The more that is learned about mycorrhizae the more it becomes apparent that they play a vital role in tree growth and forest ecosystems. They are an integral part of trees and their associated soils; when a tree is uprooted, a large part of its root system, a functional part, is left behind as a web of fungal hyphae. Trees cannot be regarded in isolation from their fungal symbionts if the forest is to be managed effectively on a biological basis.

Although there are wide gaps in our knowledge, and some conflicting evidence, it is clear that mycorrhizae can substantially increase the growth and survival of young trees and directly, or indirectly, benefit older trees after planting in the field when used in a biologically sound manner. At the same time, they confer many other benefits to forest ecosystems.

## The Biology of Mycorrhizae

The biology of mycorrhizae was discussed to some extent earlier in the section on the benefits of mycorrhizae. Important aspects, such as phosphorus uptake, are discussed in more detail below.

Phosphorus. Improved uptake by mycorrhizal plants of elements such as phosphorus, nitrogen, potassium, calcium, magnesium, zinc and copper is well documented. The hyphae, in association with soil bacteria, seem to be more able to solubilize inert phosphorus compounds than uninfected host roots, and increase the plants effective root system by penetrating soil microsites not otherwise accessible to the plant. The fungus uses the energy from the host plant's carbohydrates to acquire soil nutrients more efficiently than the host's roots *per se*.

The improved uptake of phosphorus was described earlier, and some increases in phosphorus uptake (+ 1464) are spectacular and approach luxury consumption (Bowen and Rovira, 1969). Some VA endomycorrhizae, in plants such as tomato, rye grass and clover, seem to be able to obtain phosphate ions from rock phosphate and tricalcium phosphate (Powell and Daniel, 1978; Bowen and Rovira, 1969); other VA mycorrhizae (eg. *Glomus fasciculatus*) seem to have more difficulty mobilizing phosphate ions from bound phosphate (Barrow *et al.*, 1977).

The mechanisms of phosphorus mobilization by mycorrhizae are not clear. On some sites, optimum pH for absorption of phosphate ions was pH

4.5 but it still occurred at pH 3.0 (Langlois and Fortin, 1978). Phosphorus is taken up as the phosphate ($PO_4^{--}$) ion. It is known that ectomycorrhizae (eg. in Fagus) contain phosphatase enzymes and chelating hydroxy acids are released into the rhizosphere; it is possible that these agents are involved in uptake of $PO_4^{--}$ (Williams and Alexander, 1975; Johnston and Miller, 1959). Once the phosphate is in the hyphae, the phosphorus can be diffused apoplastically (in the mycelia between the cells) and some may enter the VA arbuscules. Phosphorus may also be stored as polyphosphates and phospholipids (Carlisle, 1982; Cox and Tinker, 1976; Losel and Cooper, 1979). The role of the vesicles is not clear but they may have a storage function.

Nitrogen. Improved nitrogen uptake on some sites may be due to the fact that some mycorrhizae such as ericoid (eg. on Vaccinium) and ectomycorrhizae (eg. on Pinus), appear to have a high affinity for ammonium ($NH_4^+$) ions; beech ectomycorrhizae seem to take up $NH_4^+$ ions more readily than $NO_3^-$ ions, which is an advantage on relatively acid forest soils where $NO_3^-$ can be relatively scarce (Hatch, 1937; Stribley and Read, 1976; Carlisle, 1982). Nitrogen levels are higher in nodulated legumes inoculated with VA endomycorrhizae than in uninfected plants (Moss et al., 1976; Smith and Daft, 1977). This may be partly due to the mycorrhizae-induced, improved nutrition of the plant which increases the size of the Rhizobium/legume nodules. However, there may be more direct fungus/Rhizobium interactions; the position is not clear. Mycorrhizae occur on many non-leguminous, actinorhizal trees (Rose, 1980), but the interactions of the multiple symbioses are not fully understood. Rose and Youngberg (1981) found VA mycorrhizae (Glomus gerdemannii) induction on actinorhizal Ceanothus velutinus Dougl. considerably improved growth, nodulation and nitrogenase activity. Improved phosphorus uptake by mycorrhizae, and possible storage as phospholipids, is likely to affect the nitrogenase activity's energy suply (ATP), either directly or indirectly.

Ericoid endomycorrhizae are associated with plants such as Calluna, Erica and Vaccinium growing on soils where available nitrogen in $NH_4^+$ or $NO_3^-$ forms is very low. It appears that on these sites, ericoid mycorrhizae enable the plants to take up nitrogen in the organic amino acid form. This requires more research (Read, 1981).

Carbohydrates. Within higher plants, a great deal of the assimilated carbon is translocated to the mycorrhizal fungus as relatively simple sugars such as glucose, fructose and sucrose. These are converted by the fungus into glycogen and insoluble lipids or phospholipids not available directly to the host (Harley, 1969). It is likely that there is a two-way exchange of sugars between the host and the fungus, with some of the host carbohydrates normally exuded from the roots into the active rhizosphere populations (including pathogens) being used by the fungus. Some fungi cannot exist as saprophytes but seem to be obligate biotrophs i.e. they must live associated with higher plants. They rely on the hosts for supplies of simple sugars since they seem to be unable to decompose complex polysaccharides in dead leaf and wood litter due to the absence, or low levels, of the enzyme cellulase. Other fungi can exist saprophytically or biotrophically (Hacskaylo, 1973; Rowell, 1938; Norkrans, 1950; Carlisle, 1982).

Competition. Mycorrhizae, like all other living organisms, have their predators, such as nematodes (eg. Aphelenchus and Meloidogyne) (Riffle, no date; Sutherland and Fortin, 1968). The fungi have to survive in circumstances of severe competition, particularly on poor soils where nutrient supplies are limited. Mycorrhizae themselves compete with other organisms and may have a profound effect upon soil microorganisms involved in litter decomposition, including the mineralization and nitrification processes.

Romell (1935, 1938) noted major effects in forests from trenching and isolating areas from living host tree roots and their associated mycorrhizae. More recent work in radiata pine stands indicates that the presence of mycorrhizal fungi suppresses litter decomposition and could be a major factor in the formation of raw humus. Such an effect could be a natural control of excessive leaching and volatilization of nitrogen, and may even contribute to the stability of climax communities in temperate regions (Gadgil and Gadgil, 1974).

The deep organic matter in many woodlands of the boreal forest contains large amounts of mycorrhizal hyphae, and litter breakdown and nutrient release is very slow indeed. Although environmental factors (eg. temperature) play a major role in retarding litter decomposition in the boreal region, it is possible that the regulatory effect of mycorrhizae is also important. Though the mechanisms of the process are not clear, it has been shown that antibiotics are produced by mycorrhizae (Krywolap et al., 1964; Marx and Davey, 1969 a, b) and these may be involved.

The biology of the mycorrhizal association is, like any symbiosis, very complex due to the host/symbiont interaction. In spite of much research little is known, but empirically it is clear that mycorrhizae are usually beneficial. Much more knowledge is needed so that mycorrhizae, either in the duality of host/fungus symbiosis, the triple interaction of fungus/host/and nitrogen-fixing organisms, or the quadruple symbioses where both endo- and ectomycorrhizae are involved, can be manipulated to improve tree yield and survival, and possibly regulate litter decomposition and nutrient release. The reader is referred to the reports by Smith (1980), Meyer (1974), Safir (1980), Harley (1969), Cooke (1977), Moss (1973), Sanders et al. (1975), and Marks and Kozlowski (1973) for more details.

## Handling Mycorrhizal Symbionts

Trees can be inoculated with mycorrhizae in many ways. Any soil that has supported trees within the last century is very likely to contain a wide range of fungi capable of forming endo- or ectomycorrhizae. Forest soils over most of the world contain many common fungi, and Wilde (1968) reports that trees on prairie soils (with no forest microflora) were successfully inoculated with mycorrhizae by forest soils from New Zealand, India, Italy, Alaska and Wisconsin.

If an uninoculatd ectomycorrhizal tree is planted in a forest soil it is likely to become inoculated eventually by the native microflora. This is not the case on prairie and other grassland soils which have not supported ectomycorrhizal trees in historical times; these soils do not contain ectomycorrhizae. Even if ectomycorrhizal spores blow onto these grassland soils, the fungi soon die out since there are no hosts. Trees

planted on these soils will generally perform very poorly without mycorrhizal inoculation and this accunts for many failures (Iyer et al., 1980).

The disadvantage of relying on the natural inoculation of planted tree seedlings is that even on forest soils fungal inoculation is unpredictable since many species of Basidiomycetes, Gasteromycetes etc. can form either effective or ineffective ectomycorrhizae. The rate and extend of inoculation is also hard to forecast. The inoculation may be delayed while the mycorrhizae compete with other soil organisms including the soil-borne pathogens which could gain early, unimpeded, access to young uninoculated plants. it may deny the young plant the mycorrhizal benefits when it needs them most.

The crudest method of inoculation is to scatter forest soil known to contain hyphae on areas to be planted that have not carried trees before (eg. grassland), or in nurseries, or in container media. This can be done, at great cost, on grassland soils at planting time. Planting should not be delayed after scattering soil inoculum on grassland sites or, lacking hosts, the mycorrhizae are not likely to survive for long. However, the identity of the fungal inoculants or the rate of infection will not be controlled. This method does not exclude the possibility of introducing nematodes and pathogenic fungi.

This method tends to be "hit or miss", and pre-inoculation of seedlings by a selected, suitable fungus is to be preferred since it gives more control and security. The inoculation of trees follows the sequence illustrated in Figure 1. There are, however, major problems in mass production of endomycorrhizae which do not culture or store readily. The methods of handling endomycorrhizae will be discussed briefly and methods for ectomycorrhizae will be given in more detail.

Endomycorrhizae

Although it is known that endomycorrhizae benefit many plants, including trees, on both fumigated and non-fumigated soils, there are difficulties associated with their handling that have impeded their utilization on a big scale. In general, the aseptate Phycomycetes, which include many of the Va endomycorrhizae of value to farmers, horticulturists and foresters, are difficult to culture and freeze dry for storage.

Fungi such as Endogone cannot be grown in synthetic media like the more amenable ectomycorrhizae. The production of endomycorrhizal inoculum can, however, he carried out in several ways in the presence of host tissue. Mixed inocula, consisting of pieces of infected host roots, mycelia and extramatrical spores, cn be obtained by sieving mixtures of infected soil and/or infected plant roots, or by raising inocula of desired strains on host plants grown in aseptic media (Crush and Pattison, 1974).

Although these inocula are fairly simple to obtain, it is less easy to store them. Some people store cultures by freeze-drying but inoculum survival is variable. There have been some successes, however, and the endomycorrhizal fungus (Glomus mosseae) on young soybean roots, frozen (-20°C) and dried in a vacuum and ground in a twenty-mesh mill, was

successfully applied as a suspension in water (Jackson et al., 1972). Crush and Pattison (1974) found that spore-containing sievings and infected roots survived freezing and drying. They also had some success with pelleting the endophytes.

In research on endomycorrhizal forest trees, valuable trees such as Liquidambar, Acer, Platanus, etc. have been successfully inoculatd with fungi such as Glomus mosseae, G. caledonius, G. microcarpus, etc. The techniques involve applying to young trees in boxes or other containers, chopped roots of infected Glycine spp., either alone or mixed with soil, or by root washings of infected roots (Melichar et al., 1982; Bryan and Reuhle, 1974; Schultz et al., 1981). The media in which the trees are grown can be sterile or non-sterile prior to inoculation, depending upon costs and objectives.

There is often some difficulty identifying endomycorrhizal fungi since some of them do not produce fruiting bodies (as the ectomycorrhizae produce typical mushrooms). However, infection can readily be confirmed by staining and observation of typical aseptate VA with arbuscules, vesicles and chlamydospores.

Most of these methods have been applied in research conditions, and large-scale production of pure endomycorrhizal inocula is still a major problem even for agricultural and horticultural crops which depend upon introduced endomycorrhizae on chemically-fumigated soils.

Ectomycorrhizae

Compared with endomycorrhizae, the culture and application of ectomycorrhizal fungi presents fewer problems. The ectomycorrhizae have hyphae and spores that can be used as inocula as in the endomycorrhizae, but they also have extensive rhizomorphs and relatively large fruiting bodies as additional sources of inocula. Furthermore, at least some of the ectomycorrhizal fungi (eg. Pisolithus tinctorius) can be cultured without too much difficulty. Nevertheless, there are still many problems to be solved in the mass culture of ectomycorrhizae and the mass inoculation of forest trees.

Large-scale inoculation of forest nurseries with ectomycorrhizae throughout the world has been carried out for many years. Methods of nursery soil inoculation used in the past include:

(i) importation of soil from the forest or old tree nurseries

(ii) raising seedlings in containers with a substrate inoculated with mycorrhizal fungi and planting them in the nursery

(iii) distribution of pure cultures of fungi

(iv) accidental inoculation by spores from nearby forest (after Mikola, 1973)

(v) distributing pieces of fruiting bodies (sporocarps)

The problem with importing quantities of soil containing mycorrhizal inocula is that it is costly and time consuming. When the forests are distant from the nursery this is impracticable. The distribution of large

quantities of mycorrhizal inocula, whether as pieces of sporocarps, hyphae or rhizomorphs needs a great deal of inoculum and is time consuming; also, it may take a long time for the inocula to spread throughout the nursery. The importation of inoculated trees appears to be the more economical and practical option for nurseries if there are the facilities to raise the trees in inoculated containers.

In recent years, huge numbers of trees have been raised in containers in greenhouses and, after a period of hardening-off in shade-halls, have been planted directly into the field, by-passing the nursery with various degrees of success. The increasing use of containers provides a good opportunity to mass inoculate the plants in the greenhouses but also raises other problems such as the response of the mycorrhizal fungi to the conditions in the container buildings, fertilizers, biocides, and possible cold storage.

When deciding upon suitable fungal symbionts to use, it is necessary to take into account their physiology and mineral nutrition (Bowen, 1973) and evidence of past performance, if any. The selected species must then be isolated, purified in successive cultures and confirmed taxonomically. Without a fruiting body this is not always easy, and ectomycorrhizae do not produce mature sporocarps on synthetic media.

The final selection of the symbiont should be based on its physiology, viability, infectivity, nutritional efficiency, rate of growth, tolerance of handling, tolerance of soil conditions and competitive ability, as well as its ability to protect the host against soil-borne pathogens. This information can be obtained by microbiological techniques that will not be discussed here.

Culture and Inoculation. Standardized techniques have been developed to raise pure cultures of ectomycorrhizal fungi (Figure 1) such as Pisolithus tinctorius whose physiology is fairly well known (Marx and Bryan, 1975). These techniques can be modified for individual fungi since their requirements and tolerances vary.

In Marx's technique (Marx and Bryan, 1975) the fungal starting inoculum (Pisolithus tinctorius) was grown at 25°C for three weeks on plates of modified MMN (Modified Melin-Norkrans agar medium) with the sucrose replaced by glucose.

The inoculum was mass-produced in 2 litre glass jars with metal, threaded lids using a medium of 1450cc of Grade 2 vermiculite, 50cc of finely divided peat moss, and 750cc of liquid MMN medium. One millimetre thick plastic discs, with 6 x 2 cm cotton-packed pyrex tubes passing through their middle that were sealed with a heat resistant, silicone rubber compound (RTV - 102, G.E.C.), were placed in the metal, threaded lids of the jars and sealed with RTV - 102. The jars were autoclaved for thirty minutes and then inoculated with eight 88 mm diameter agar discs containing the mycorrhizal hyphae. The lids of the jars were wrapped with parafilm, and kept at room temperature.

The fungal mycelia had permeated the vermiculite after fifteen weeks and were ready for mass inoculation. The mycelium-infected vermiculite was passed through a 5 mm mesh sieve and leached on cheese cloth with 8

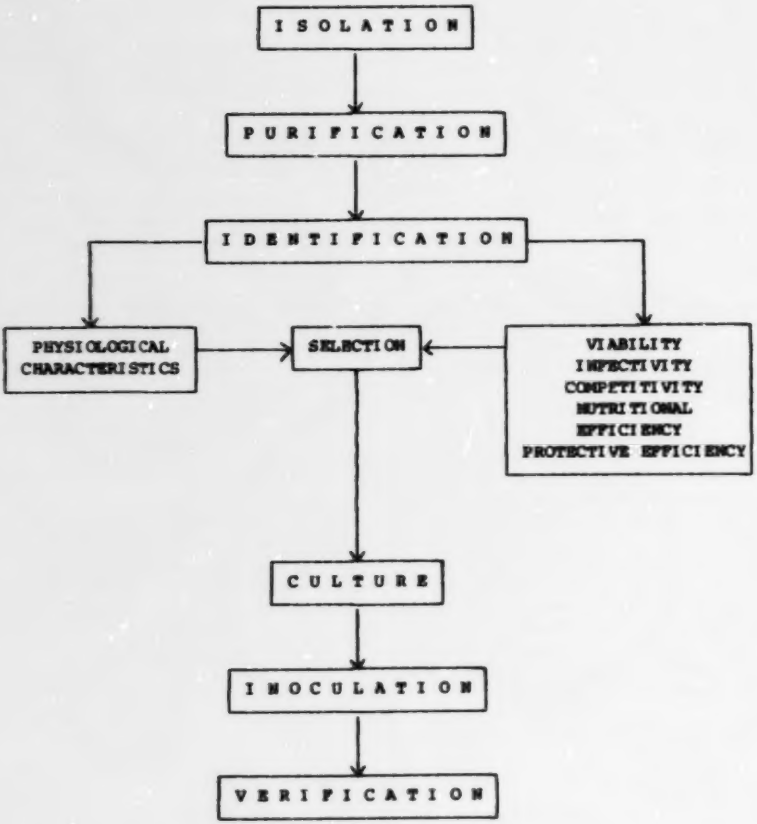


Figure 1. Steps in inoculation with mycorrhizal fungi

(after Fortin, J.A. and M. Pineau, 1971)

litres of cool tap water to remove excess nutrients, prevent excessive development of saprophytes, and reduce the risk of damping off. The leached inoculum was placed in plastic bags and refrigerated for twenty-four hours before use (Marx and Bryan, 1975).

These cultures can be raised in small glass vessels for research work, or in larger glass vessels and plastic bags for more extensive work. Production of inocula for forestry operations is carried out in industrial fermentors, as in the Southern Pine Program in the United States. Cultures should not be left for long in cold storage prior to use unless the humidity content is lowered about 16%.

Vermiculite is expensive, and other media, such as lignite and tree bark are being tested for inoculum production with various degrees of success.

Tree inoculation is the point at which the forester can take over from the microbiologist. The aim is to get the symbiotic fungus into the plant as soon as possible. The usual method is to mix the vermiculite with the medium in which the trees are raised (eg. Marx et al., 1982).

Recently, attempts have been made to incorporate the mycorrhizae in pelleted seed in a manner similar to that with nitrogen-fixing Rhizobium inocula, or just into pellets to be scattered like fertilizer. Commercial pelleted seeds containing mycorrhizae are available, but the period the fungi remains viable is questionable. Rhizobium pellets require use soon after coating, and it is possible the same applies to the fungi. Crush and Pattison (1974) found that the endomycorrhiza Glomus mosseae could be pelleted, and sowing the host seeds above Glomus-infected pellets gave better results than infected seed pellets. Inoculation by spore-containing pellets of either ecto- or endomycorrhizae is being investigated.

For the present time, however, foresters will need to rely on more conventional inocula such as:

i) pure cultures (for ectomycorrhizae)

ii) mycorrhizal roots (especially for endomycorrhizae)

iii) spores

iv) sporocarps (for ectomycorrhizae)

v) mycelia in a soil medium (vermiculite, peat, powdered lignite, etc.)

vi) mycelia in liquid suspension

vii) sclerotia

viii) rhizomorphs (ectomycorrhizae) (after Langlois and Fortin, 1981)

Pure cultures are preferable where available and great efforts are being made to upgrade inoculum quality.

Use in Containers. Lists of trees and mycorrhizal fungi tested in containers are given in Tables 13 and 14. The tree list is not exhaustive, and there are many other (probably over 2000) ectomycorrhizal fungi capable of forming effective mycorrhizal associations. The fungi are crudely ranked for known success, but these are only for the conditions under which they were tested. Some fungi, such as Pisolithus tinctorius, appear to be very plastic; Pisolithus has been effectively used in the Southern Pine Program in the United States and its use has reached the stage of mass, industrial production. Nevertheless, it is strongly suggested that foresters test and develop fungal symbionts which perform well on various sites in the locality where the trees will be grown.

During culture and the post-inoculation period, it should be remembered that with ectomycorrhizae:

(i) mycorrhizal development is negatively affected by light intensities less than 50% daylight on the seedlings

(ii) most mycorrhizal fungi operate best at pH's of 4.0 to 5.0

(iii) most fungal strains grow well between 15°C and 30°C

(iv) a medium aeration level of at least 15% should be maintained; below 5% they will not grow as well

(v) the water in the substrate should be 40% to 50% of field capacity

(vi) mycorrhizae respond well to some nutrients but excessive application of fertilizer (eg. nitrogen) can be detrimental

(vii) mycorrhizae respond adversely to many biocides used to control soil pests and diseases in nurseries and greenhouses (Table 15) (after Langlois and Fortin, 1981)

## Mycorrhizae in Intensive Forestry

Mycorrhizae are of great potential value to foresters for the production of nursery stock and container plants. The benefits of mycorrhizae to the trees overshadow their occasional drawbacks. The advantages have already been discussed but, in brief, mycorrhizae confer to trees better nutrient uptake, resistance to drought and disease, and better growth. The network of hyphae extending from the roots are as much a part of the tree as its other parts, but are usually ignored except where mycorrhizae have been used successfully, such as in the Southern Pine Program in the United States.

Although the selection, screening, culture and inoculation of mycorrhizae pose many problems, few, if any, are insurmountable. The use of container stock has provided new opportunities for application of mycorrhizal techniques, although the container approach to reforestation per se has encountered many problems, such as root distortion, frost lift, and low survival. These still need to be overcome.

Table 13: Main Trees Tested in Containers in Europe and North American[1]

| Mycorrhiza | Tree Species | Mycorrhiza | Tree Species |
|---|---|---|---|
| ECTO [2] | Cedrus atlantica | ECTO | Pinus palustris |
| ENDO [3] | Liquidambar styraciflua | " | P. pinaster |
| | | " | P. ponderosa |
| ECTO | Picea abies | " | P. rigida |
| " | P. engelmannii | " | P. silvestris |
| " | P. glauca | " | P. taeda |
| " | P. mariana | " | Pseudotsuga menziesii |
| " | P. sitchensis | | |
| " | Pinus banksiana | " | Quercus alba |
| " | P. echinata | " | Q. macrocarpa |
| " | P. elliottii | " | Q. palustris |
| " | P. flexilis | " | Q. robur |
| " | P. halepensis | " | Q. rubra |
| " | P. nigra | " | Q. velutina |
| | | " | Tsuga heterophylla |

[1] Work has also been done with mycorrhizal container stock with tropical pines (eg. Pinus caribea (ECTO)), and with fast-growing Pinus radiata in Australasia

(from: Langlois and Fortin, 1981)

Table 14: Main Ectomycorrhizal[1] Fungi Tested in Container Programs

| | | Fungus Species |
|---|---|---|
| | | Agaricus sylvaticus |
| | | Amanita pantherina |
| | | Astraeus pteridis |
| | | Boletinus cavipes |
| 2 | *** | Cenococcum geophilum |
| | ** | Hebeloma crustuliniforme |
| | * | H. cylindrosporum |
| | *** | Laccaria laccata |
| | | Lycoperdon gemmatum |
| | ** | Pisolithus arhizus |
| | **** | P. tinctorius |
| | ** | Rhizopogon luteolus |
| | | R. roseolus |
| | | Sphaerosporella brunnea |
| | | S. columnare |
| | ** | Suillus granulatus |
| | | S. luteus |
| | * | S. tomentosus |
| | *** | Thelephora terrestris |

1 The endomycorrhizal fungi are not listed because few have been tested in container programs. Liquidambar and Acer have been successfully inoculated with Glomus spp. and these fungi are the principal endophytes used, eg. Glomus mosseae, G. microcarpus, G. fasciculatus, G. etunicatus, etc.

2 The number of asterisks gives a crude indication of degree of success in some programs (eg. * = some success, and **** = very successful). However, this does not mean the highly-rated fungi will be successful in all circumstances.

(from: Langlois and Fortin, 1981)

Table 15: Effects on Ectomycorrhizae of Biocides Used in Forestry

| | Effect [1] |
|---|---|
| **Fumigants** | |
| Methyl bromide | - |
| Vapam | - |
| **Fungicides** | |
| Arasan | + |
| Banrot | + |
| Bayleton | + or - |
| Benlate (benomyl) | + or - |
| Benodanil | - |
| Captan | + or - |
| Dexon (fenaminosulf) | - |
| Murtect (thiabendazole) | 0 |
| Terrachlor (quintozene) | - |
| Truban (ethazole) | 0 |
| **Insecticides** | |
| Lindane | - |
| Toxaphene | - |
| **Herbicides** | |
| -2 -4 -D, simazine | - |

[1] 0 no effect on mycorrhizal fungi

\+ = positive effect

\- = negative effect

(from: Langlois and Fortin, 1981)

Foresters are well aware of the long-term advantages of using healthy, relatively large trees as planting stock. Mycorrhizae can help in this respect, providing plants with high competitive ability.

Both biocides and fertilizers are used in forestry, particularly in nurseries. Mycorrhizae respond in different ways to different biocide and fertilizer treatments (Table 15), and this will need to be taken into account by forest managers (Pawuk et al., 1980; Pawuk and Barrett, 1981). It would be unfortunate if inocula were cultured, and the trees successfully inoculated, only to have the mycorrhizae inhibited by antagonistic chemicals.

Mycorrhizae could have a significant impact on fertilization costs. In some parts of North America, nitrogen fertilizers are used extensively in forestry, but not a great deal of phosphorus fertilizer is used. In Europe, phosphorus fertilizers have provided good tree growth responses and are used more widely. Many forest soils have low levels of available phosphorus, and the income of this element in precipitation is very small (less than 1 kg $ha^{-1}$ $year^{-1}$). Phosphorus fertilizers include, for example, ground rock phosphate and calcium-containing superphosphates, but these are becoming scarce and costly. If trees are inoculated with effective mycorrhizae they can utilize relatively inert phosphates, and efficiently utilize the phosphorus that is available, greatly reducing the quantities of phosphorus fertilizer needed. This is particularly the case in agriculture where use of mycorrhizae can substantially reduce the fertilizer costs on arable land, and this benefit would apply when mini-rotation forests of poplars, for example, are grown on formerly arable land.

Since mycorrhizae appear to inhibit decomposition of soil organic matter, when this process is better understood the forester may be able to use the mycorrhizae as a soil management tool.

When forests are harvested there is often a rapid mineralization of soil organic matter due to many factors (altered microclimate, aeration, etc.). This stimulates the ammonification and nitrification processes, with nitrogen and other nutrients becoming mobile and being lost by leaching or as gases. It is possible that mycorrhizal hyphae, which inhibit litter decomposition on some sites may play a key role in controlling this process; by harvesting the tree hosts, the hyphae's inhibiting effects could be reduced which would allow decomposition to accelerate. The decomposition of the dead hyphal mats could also release carbohydrates and provide an energy-rich substrate and stimulate soil organisms. A forester could, by manipulating the tree cover, maintain enough hyphae following felling to reduce organic matter losses.

Conversely, the forester could inhibit or reduce mycorrhizal populations and thereby reduce excessive organic matter build up and release locked-up nutrients if temperature and other environmental factors permit adequate soil microorganism activity. Mycorrhizal populations are sensitive to light and biocides and could be manipulated silviculturally.

Mycorrhizae have the potential to improve young tree growth and survival and provide a soil management tool. Whether or not these attributes are utilized will largely depend upon whether or not foresters

appreciate their value, want to use them, and request more information from forest scientists.

If foresters are to take full advantage of mycorrhizae, not only is much more knowledge on their biology and performance needed, but there is also a need for foresters to take mycorrhizae and the other soil biota into account in their management plans.

In general, foresters are not well-informed about soil biology. Forestry schools teach their students about soil classification, site selection, soil structure and at least a little about soil physics and chemistry. However, the soil biota are seldom given more than a brief mention. Until this gap is filled and foresters are better informed, they are not likely to take advantage of mycorrhizae or other microbiological techniques of tree and soil management.

## DIRECTORY OF RESEARCHERS

In the past six years, work on both nitrogen-fixing and mycorrhizal symbioses has greatly increased. It is recognized by many that these symbioses are at the frontier of our knowledge and that when we learn to use them effectively they can be of great benefit. The number of researchers in this field is increasing rapidly and the following directories are available:

1. Rottinck, B.A. 1980. International Directory of Researchers in Biological Nitrogen Fixation. Crown Zellerbach. Special Publication No. 1: 67 pp. (deals mainly with forestry applications).

2. Furlan, V. and J.A. Fortin. 1981. First International Directory of Mycorrhizae Researchers. Dept. des Sciences Forestières, Faculté de Foresterie, Université Laval, Québec. 103 pp. (deals with forestry, agriculture and horticulture).

## FUTURE ACTIVITY

It is suggested that in the future, research, field testing, and education in the potential uses of nitrogen fixation and mycorrhizae in forestry should be carried out as follows:

### Education

(a) More emphasis should be given to soil biology and soil biome-higher plant interaction in forestry school curricula to provide the forester with an information base on nitrogen fixation, mycorrhizae and microbial processes involved in nitrogen and phosphorus transformations in the soil.

(b) Foresters should be encouraged to think of silviculture in terms of both crop and soil management, and to take into account the interaction of trees, nitrogen-fixing soil bacteria and mycorrhizal fungi in their plans for crop establishment, management, and harvesting. Prescriptions for the use of biocides and fertilizers should take into account the response of the soil biota.

## Nitrogen Fixation

(a) Well-designed experiments should be carried out specifically to develop and test the effects of using nitrogen-fixing organisms in intensive forestry. These experiments should include the development of silvicultural systems involving either crop rotations or mixed plantations incorporating trees with nitrogen-fixing symbioses (legumes and non-legumes) to improve soil fertility and fibre yield.

(b) The actinomycetes involved in actinorhizal, nitrogen-fixing symbioses should be screened to find superior symbionts and the most suitable hosts for use in forestry.

(c) The genetics of Alnus spp. should be investigated; the performance of different species and provenances in different locations and select superior genotypes should be tested. Plastic, fast-growing types with arborescent habits suitable for use in forestry on many sites should be created and problems of host/actinorhiza interaction should be clarified.

(d) The optimal condition for nodulation, actinorhizal nitrogen fixation and host/actinorhiza growth and activity should be examined to assist in the selection of types useful in forestry.

(e) The banks of actinorhizal symbionts (eg. Frankia) with different characteristics from a wide range of hosts should be increased to provide material for researchers and foresters.

(f) Methods should be found to transfer the nitrogen-fixing organisms to roots of other tree hosts of commercial value so that they can fix nitrogen within the new hosts by overcoming the infection barrier by genetic or somatic manipulation of host and actinomycete.

(g) More about the ecology of nitrogen fixation and its distribution in the forests should be learned.

## Mycorrhizae

(a) Mycorrhizal symbioses between fungi and trees of potential value in intensive forestry should be investigated. Very little is known about mycorrhizae in species of Salix, Populus and Acer, all of which have promise in short-rotation, high-yield systems.

(b) Mycorrhizal symbionts should be screened and tested on hosts in order to find effective, plastic, aggressive symbionts capable of competing with wild fungi, etc. in forest soils. It would be unwise to rely on Pisolithus for all situations, and better to select or develop symbionts suitable for local use.

(c) Cultural inoculation techniques should be developed which are specifically designed for selected mycorrhizal symbionts and ways should be developed to evaluate them.

(d) Better nursery techniques involving the use of mycorrhizae on a wide range of tree species should be developed.

(e) The interactions between the hyphae of mycorrhizal fungi and the soil biota involved with litter decomposition should be investigated to find out whether or not mycorrhizae can be used to regulate soil organic matter decomposition and nitrogen availability in forests.

(f) The biology of mycorrhizae should be investigated in order to better understand the processes of nutrient and water uptake, storage of carbohydrate and lipids, and host/symbiont exchange of water and energy, antagonism, and protection of hosts from soil-borne pathogens.

### Both Nitrogen-Fixing and Mycorrhizal Symbioses

(a) Rapid methods should be developed to evaluate the natural inoculum potential of the sites before planting.

(b) Strains should be selected that are adapted to alders, willows and poplars.

(c) Rapid laboratory methods should be developed to evaluate the efficiency of symbionts.

(d) The receptivity of the tree clones produced by tree breeders should be evaluated.

(e) The most efficient isolates for competitiveness and adaptation to sites used for energy plantations should be tested in the field.

(f) The inoculum technology for energy plantations should be adapted.

(g) The indirect effects of biocides and fertilizers on the formation and functioning of the symbioses should be evaluated.

(h) Special attention should be given to vesicular-arbuscular endomycorrhizae and rapid transfer to forest trees of the knowledge acquired on agricultural crops should be assured.

## CONCLUSIONS

It is concluded that nitrogen-fixing and mycorrhizal symbioses have considerable potential for use in forestry to increase the quality, size and survival of young trees used in planting programs, shorten rotations, increase fibre yield, and enable management of the soil to maintain long-term site productivity with reduced use of costly chemical fertilizers.

The use of these symbioses is still in its infancy, and there are large gaps in our knowledge about the fundamental processes of the symbioses and how to use them effectively in intensive forestry systems.

Carefully designed field tests and indepth biological research are needed if effective nursery and container practices and silvicultural systems are to be developed.

Nitrogen-fixing and mycorrhizal symbioses are additional, low-energy tools at the forester's disposal for managing the forest; they can supplement the array of other tools (fertilizers, biocides, vegetation manipulation). Even when the symbioses are used in intensive forestry, it will still be necessary to use some chemical fertilizers to maintain nutrient levels, but it is anticipated that less of these costly chemicals will be needed. Biocides will have to be used with discretion, keeping in mind the soil biome as well as the vegetation.

Many operational foresters are not sufficiently informed about soil biology to take full advantage of these symbioses even where techniques exist, and forestry schools need to place greater emphasis on this subject in their cirricula. If symbioses are used, it will be necessary for foresters to accept that forest plant species that do not produce commercial fibre are not necessarily "weeds", but can have potential for soil management. New attitudes will be needed as well as new techniques.

## ACKNOWLEDGEMENTS

The authors wish to thank the numerous people who helped with this report, particularly Dr. Maurice Lalonde, Dr. Lake Chatarpaul, Mr. Darwin Burgess, Mrs. Joan Carlisle and Ms. Susan E.A. Carlisle.

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## APPENDIX I

### Trees of Potential Value in Intensive Forestry; Their Nitrogen-fixing and Mycorrhizal Symbioses

In their International Energy Agency report on tree species suitable for intensive forestry, Lavoie and Vallee (1981) provided a list of tree species with fibre production potential. The list did not include trees and shrubs with soil amelioration potential, some of which are described in this report.

In Appendix I, species are listed where specific information was located in the literature that they have rhizobial, actinorhizal, endomycorrhizal or ectomycorrhizal symbioses. The list is, of course, incomplete. Some of the information located was only at the genus level; other sources referred directly to species. Where there is evidence that several other species (not necessarily listed in this Appendix) form symbiotic relationships, and it is very likely that all species within the genus have similar symbioses, the symbiosis code is bracketed (eg. (ECTO), (ENDO), (ACN), or (RH)). This just means that although specific information sources were not located, these symbioses are likely. For example, in Pinus, Larix and Abies genera all the species are very likely to be ectomycorrhizal. Not all Alnus species have been studied, but they are all probably actinorhizal and mycorrhizal.

It is safe to predict that, ultimately, all the species listed will prove to be mycorrhizal to some extent when exposed to compatible inocula.

LEGEND

A. NITROGEN-FIXING SYMBIOSES

| | | |
|---|---|---|
| RH | = | Rhizobial (*Rhizobium*) |
| ACN | = | Actinorhizal (*Frankia*) |
| AR(L) | = | A legume almost certainly with a *Rhizobium* symbiosis even though a specific literature reference was not listed |
| ( ) | = | Likely has nitrogen-fixing symbioses |

B. MYCORRHIZAL SYMBIOSES

| | | |
|---|---|---|
| ECTO | = | Ectomycorrhizal |
| ENDO | = | Endomycorrhizal (Vesicular Arbuscular in all cases) |
| ECTO AND ENDO | = | Both |
| ECTENDO | = | Ectendomycorrhizae |
| ( ) | = | Likely has mycorrhizae |
| ? | = | Information not located |

Nitrogen-fixing and Mycorrhizal Symbioses Associated

with Trees of Potential Value in Intensive Forestry

| TREE GENERA AND SPECIES | SYMBIOSES $N_2$ - Fixing | Mycorrhizae | REFERENCE |
|---|---|---|---|
| **ANGIOSPERMS** | | | |
| Acacia spp. | RH | (ENDO) | Burns and Hardy (1975) |
| A. confusa | RH (L) | (ENDO) | |
| A. cyanophylla | RH (L) | (ENDO) | |
| Acer spp. | - | ECTO+ENDO | Meyer (1973); Schultz et al. (1981) |
| A. negundo | - | (ENDO) | |
| A. pennsylvanica | - | (ENDO) | |
| A. pseudoplatanus | - | ENDO | Walker and Bartlett (1981) |
| A. rubrum | - | (ENDO) | |
| A. saccharinum | - | ENDO | Spitko et al. (1978) |
| A. spicatum | - | (ENDO) | |
| Ailanthus spp. | - | ? | |
| A. altissima | - | ? | |
| Alnus spp. | ACN | ECTO+ENDO+ ECTENDO | Virtanen and Miettinan (1963); Meyer (1973); Mexal (1980) |
| A. cordata | ACN | (ECTO) | |
| A. crispa | ACN | ECTO | Fortin et al. (1982) |
| A. glutinosa | ACN | ECTO+ENDO+ ECTENDO | Walker and Bartlett (1981); Tarrant and Trappe (1971) |
| A. hirsuta | (ACN) | (ECTO) | |
| A. incana | ACN | ENDO+ECTO | Rose (1980) |
| A. oblongifolia | (ACN) | (ECTO) | |
| A. rhombifolia | (ACN) | (ECTO) | |
| A. rubra | ACN | ENDO+ECTO | Rose (1980); Tarrant and Trappe (1971) |
| A. rugosa | ACN | ECTO | Fortin et al. (1983) |
| A. serrulata | ACN | (ECTO) | Tarrant and Trappe (1971) |
| A. sinuata | (ACN) | ENDO | Rose (1980) |
| A. tenuifolia | ACN | ECTO | Fleschner in Fortin (1979) |
| Betula spp. | - | ECTO | Meyer (1973); Mexal (1980) |
| B. papyrifera | - | ECTO | Fortin (1968) |
| B. pubescens | - | (ECTO) | |
| B. verrucosa | - | ECTO | Mason et al. (1977) |
| Caragana spp. | RH (L) | ? | |
| C. arborescens | RH (L) | ? | |

(genera and species after Lavoie and Vallée, 1981)

| TREE GENERA AND SPECIES | SYMBIOSES $N_2$ - Fixing | Mycorrhizae | REFERENCE |
|---|---|---|---|
| Castanea spp. | - | ECTO | Zak (1973) |
| C. dentata | - | (ECTO) | |
| C. sativa | - | (ECTO) | |
| Catalpa spp. | - | ? | |
| C. bungei | - | ? | |
| Eucalyptus spp. | - | ECTO | Chou-Chou (1981) |
| Fraxinus spp. | - | ENDO | Schultz et al. (1981) |
| F. americana | - | (ENDO) | |
| F. excelsior | - | ENDO | Linneman (1972) |
| F. pennsylvanica | - | (ENDO) | |
| Gleditsia spp. | RH (L) | ? | |
| G. sinensis | RH (L) | ? | |
| G. triacanthos | RH (L) | ? | |
| Lespedesia spp. | - | ? | |
| Leucaena spp. | RH (L) | - | |
| Liquidambar spp. | - | ENDO | Kormanik et al. (1977) |
| L. styraciflua | - | ENDO | Filer and Toole (1966) |
| Liriodendron spp. | - | ENDO | Gerdemann (1965) |
| L. tulipifera | - | ENDO | Schultz et al. (1979) |
| Nothofagus spp. | - | ECTO | Meyer (1973) |
| N. alpina | - | ? | |
| N. obliqua | - | ? | |
| N. procera | - | ? | |
| Paulownia spp. | - | ENDO | Reckjord (1981) |
| P. albiphloea | - | ENDO | Reckjord (1981) |
| P. australis | - | ? | |
| P. catalpifolia | - | ? | |
| P. coreana | - | ? | |
| P. elongata | - | ? | |
| P. fargesii | - | ? | |
| P. fortunei | - | ? | |
| P. kawakamii | - | ? | |
| P. taiwaniana | - | ? | |
| P. tomentosa | - | ? | |
| Platanus spp. | - | ENDO | Schultz et al. (1981) |
| P. acerifolia | - | (ENDO) | |
| P. occidentalis | - | ENDO | Schultz et al. (1981) |
| P. orientalis | - | (ENDO) | |

| TREE GENERA AND SPECIES | SYMBIOSES $N_2$ - Fixing | Mycorrhizae | REFERENCE |
|---|---|---|---|
| Populus spp. | - | ECTO + ENDO | Meyer (1973); Vozzo and Hacskaylo (1964) |
| P. angustifolia | - | (ECTO) | |
| P. arizonica | | (ECTO) | |
| P. balsamifera | | ECTO | Malloch and Malloch (1981) |
| P. x berolinensis | - | (ECTO) | |
| P. x canescens | - | (ECTO) | |
| P. cathya | - | (ECTO) | |
| P. davidiana | - | (ECTO) | |
| P. deltoides | - | ECTO + ENDO | Vozzo and Hacskaylo (1964) |
| P. diversifolia | - | (ECTO) | |
| P. euphratica | - | (ECTO) | |
| P. fremontii | - | ECTO | Vozzo and Hacskaylo (1964) |
| P. grandidentata | - | ? | Vozzo and Hacskaylo (1964) |
| P. grandidentata x alba | - | ? | |
| P. heterophylla | - | ? | |
| P. x jackii | - | ? | |
| P. koreana | - | ? | |
| P. MacDougalii | - | ? | |
| P. maximowiczii | - | ? | |
| P. nigra | - | ? | |
| P. palmeri | - | ? | |
| P. pruinosa | - | ? | |
| P. pseudosimonii | - | ? | |
| P. sargentii | - | ? | |
| P. simonii | - | ? | |
| P. suaveolens | - | ? | |
| P. texana | - | ? | |
| P. tomentosa | - | ? | |
| P. tremula | - | ECTO | Meyer (1973) |
| P. tremula var. davidiana | - | ? | |
| P. tremuloides | - | ECTO | Fortin et al. (1983) |
| P. trichocarpa | - | ? | |
| P. usuriensis | - | ? | |
| P. wislizeni | - | ? | |
| Prunus spp. | - | ENDO | Gilmore (1971) |
| Quercus spp. | - | ECTO | Meyer (1973) |
| Q. nigra | - | (ECTO) | |
| Q. nuttalii | - | (ECTO) | |
| Q. phellos | - | (ECTO) | |
| Q. prinus | - | (ECTO) | |
| Q. rubra | - | ENDO | Grand (1969) |
| Rhus spp. | - | ? | |
| R. typhina | - | ? | |

| TREE GENERA AND SPECIES | SYMBIOSES $N_2$ - Fixing | Mycorrhizae | REFERENCE |
|---|---|---|---|
| Robinia spp. | RH (L) | ENDO | Daft et al. (1975) |
| R. pseudoacacia | RH (L) | ENDO | Daft et al. (1975) |
| R. viscosa | RH (L) | (ENDO) | |
| Salix spp. | - | ECTO + ENDO | Mayer (1973); Fontana (1962) |
| S. alba | | ECTO | Fontana (1962) |
| S. alaxensis | - | ? | |
| S. amplifolia | - | ? | |
| S. amygdalina | - | ? | |
| S. amygdaloides | - | ? | |
| S. babylonica | - | ECTO | Fontana (1962) |
| S. bebbiana | - | ? | |
| S. bomplandiana | - | ? | |
| S. caprea | - | ? | |
| S. cardiophylla | - | ? | |
| S. caroliniana | - | ? | |
| S. cheilophila | - | ? | |
| S. drphnoides | - | ? | |
| S. dasyclados | - | ? | |
| S. discolor | - | ? | |
| S. elegantissima | - | ? | |
| S. eriocephala | - | ? | |
| S. exigua | - | ? | |
| S. fragilis | - | ? | |
| S. goodingii | - | ? | |
| S. harbisonii | - | ? | |
| S. hookeriana | - | ? | |
| S. jesoensis | - | ? | |
| S. laurentiana | - | ? | |
| S. lasiandia | - | ? | |
| S. lasiolepis | - | ? | |
| S. laevigata | - | ? | |
| S. longifolia | - | ? | |
| S. lucida | - | ? | Fontana (1962) |
| S. mackenzuana | - | ? | |
| S. magnifica | - | ? | |
| S. matsudana | - | ? | |
| S. missouriensis | - | ? | |
| S. mongolica | - | ? | |
| S. nigra | - | (ECTO) + ENDO | Antibus et al. (1980) |
| S. pentandra | - | ? | |
| S. phanera | - | ? | |
| S. planifolia | - | ? | Fortin et al. (1982) |
| S. purpurea | - | ECTO | Fontana (1962) |
| S. pyrifolia | - | ? | |
| S. rhederiana | - | ? | |
| S. rigida | - | ? | |
| S. scouleriana | - | ? | |
| S. sitchensis | - | ? | |
| S. taxifolia | - | ? | |
| S. viminalis | - | ? | |

| TREE GENERA AND SPECIES | SYMBIOSES $N_2$ - Fixing | Mycorrhizae | REFERENCE |
|---|---|---|---|
| Sophora spp. | - | ? | |
| S. japonica | - | ? | |
| Ulmus spp. | - | ECTO + ENDO | Meyer (1973); Mexal (1980) |
| U. americana | - | ? | |
| U. japonica | - | ? | |
| U. macrocarpa | - | ? | |
| U. minor | - | ? | |
| U. parvifolia | - | ? | |
| U. propinqua | - | ? | |
| U. pumila | - | ? | |
| U. rubra | - | ? | |
| U. thomasii | - | ? | |
| GYMNOSPERMS | | | |
| Abies spp. | - | ECTO | Meyer (1973) |
| A. amabilis | - | (ECTO) | |
| A. grandis | - | (ECTO) | |
| Larix spp. | - | ECTO | Meyer (1973) |
| L. dahurica | - | (ECTO) | |
| L. decidua | - | ECTO | |
| L. eurolepis | - | (ECTO) | |
| L. griffithii | - | (ECTO) | |
| L. laricina | - | (ECTO) | |
| L. leptolepis | - | (ECTO) | |
| L. occidentalis | - | (ECTO) | |
| L. olgensis | - | (ECTO) | |
| L. potanini | - | (ECTO) | |
| L. sibirica | - | ECTO | Trerling (1973) |
| Pinus spp. | - | ECTO | Meyer (1973) |
| P. banksiana | - | ECTO | Langlois (1981) |
| P. elliottii | - | ECTO | Marx and Bryan (1975) |
| P. koraiensis | - | (ECTO) | |
| P. pinaster | - | (ECTO) | |
| P. radiata | - | ECTO | Smith (1980); Chou Chou (1979) |
| P. rigida | - | (ECTO) | |
| P. strobus | - | ECTO | Smith (1980) |
| P. sylvestris | - | ECTO | Robertson (1954) |
| P. tabulaeformis | - | (ECTO) | |
| P. taeda | - | ECTO | Blakeslee (1977); Tainter and Walstad (1977) |
| Sequoia spp. | - | ENDO | Harley (1969) |
| S. sempervirens | - | (ENDO) | |

POOR

QUALITY

ORIGINAL